The Journal of Laboratory and Clinical Medicine

VOL. 48 DECEMBER, 1956 NO. 6

CLINICAL AND EXPERIMENTAL

HEMATOLOGIC OBSERVATIONS IN BACTERIAL ENDOCARDITIS

ESPECIALLY THE PREVALENCE OF HISTIOCYTES AND THE ELEVATION AND VARIATION OF THE WHITE CELL COUNT IN BLOOD FROM THE EAR LOBE

GENEVA A. DALAND, B.S., LEONARD GOTTLIEB, M.D., RALPH O. WALLERSTEIN, M.D., AND WILLIAM B. CASTLE, M.D.

BOSTON, MASS.

THE presence of histiocytes in the peripheral blood of patients with bacterial endocarditis is presumably a reflection of the widespread hyperactivity of the reticuloendothelial system in response to the persisting infection.[1] Since 1907, this finding has been reported by many authors[2-16] but has not been especially emphasized in the recent American literature as a matter of diagnostic import. However, in this laboratory, the finding of histiocytes in the peripheral blood, especially in blood from the ear lobe, has led in several instances to studies that confirmed the suspicion of bacterial endocarditis. This peculiarity of the ear lobe is well documented in the European literature but is not mentioned in several recent American textbooks of hematology. The primary purpose of this paper is to present hematologic studies in 10 patients in whom the diagnosis of bacterial endocarditis was established by autopsy and in whom histiocytes were found in the peripheral blood on one or more occasions. The probable mechanism of their prevalence in blood from the ear lobe is discussed.

MATERIAL AND METHODS

In the 10 patients selected for this report the hematologic studies were made at the Thorndike Memorial Laboratory and the pathologic examinations were done at the Mallory

From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School and the Mallory Institute of Pathology, Boston City Hospital, Boston, Mass.

Received for publication May 14, 1956.

Institute of Pathology of the Boston City Hospital.* In 7 patients the diagnosis was made or at least suspected clinically. In 2 of these 7 cases, the diagnosis was first suggested by the discovery of large numbers of histiocytes in peripheral blood films. In the 3 cases which were not correctly diagnosed during life, the clinical evidence was not considered to be consistent with bacterial endocarditis, although histiocytes in the blood films were reported. In some patients the studies during life were extensive because the diagnosis was known or suspected, while in others only a few blood films had been saved because of the unusual cells which were present at a time when the diagnosis of bacterial endocarditis had not been made. In addition, special control studies of capillary and venous blood were made in 15 normal subjects and capillary counts from day to day in 2 patients with myelogenous leukemia and 4 normal subjects.

The blood samples were obtained by stylet puncture from the ear lobe, tip of the finger, or by venipuncture from the antecubital vein. These sources will be referred to hereafter simply as ear, finger, or vein. The red cells were counted by the use of 2 red cell pipettes, 1 for each side of a double Levy-Hausser counting chamber. When the white cells were counted, both sides of the chamber were used and the cells were enumerated in an area of 8 square millimeters. For routine work, 1 pipette was filled, but in the observations on the 15 normal controls, 2 pipettes were used, and the white cells from each pipette were counted in an area of 8 square millimeters. For counting the white cells of the leukemia cases, 2 red cell pipettes were used, with a dilution of 1/200, the cells being counted in 8 square millimeters for each pipette. All pipettes were guaranteed to meet the specifications for certification set by the United States Bureau of Standards. Hemoglobin was determined with the Evelyn photoelectric colorimeter. Wintrobe hematocrit and red cell indices were determined.

For making the blood films, prepared on coverslips, the source of the sample, whether from the ear, finger, or vein, was that used for the corresponding white cell count. In determining the differential white cell count, 400 white cells from each sample were classified, 2 or more coverslip preparations being used. At least 200 of these cells were classified by one of the authors (G. A. D.). Reticulocyte counts were made on other blood films in which the cells had been vitally stained with brilliant cresyl blue and counterstained with Wright's stain. Platelet counts were done on a few patients, the modification of the Reese-Ecker method being employed.[17] Osmotic fragilities were done by standard methods.[18]

The term histiocyte is used here to denote a cell which is believed to be derived from the reticuloendothelial tissue. Such cells normally may appear in very small numbers in the peripheral blood. Terms, which have been used by others, to describe the same type of cell are "clasmatocyte," "macrophage," "phagocytic monocyte," "endothelial cell," "hemohistioblast," and "large mononuclear cell." In fact, in many reports in the literature no differentiation has been made between the histiocyte and the monocyte.

The histiocyte, as seen in Wright's stained blood films from patients with endocarditis, is a cell with large irregular cytoplasm, often ameboid in appearance, with an oval, kidney-shaped, irregular, or round nucleus. The nuclear structure has a lacy or honey-comb appearance and is sometimes folded. The purple color of the nucleus with Wright's stain is usually lighter than that seen in either the monocyte or the lymphocyte. Sometimes nucleoli are present. The abundant cytoplasm is a pale gray-blue and the edge may be so delicate that the cell membrane is difficult to discern, giving the appearance of a veil. In the cytoplasm there may be vacuoles of various sizes, granules, cellular debris, phagocytosed red cells, white cells, or platelets. Some of these cells may be confused with monocytes or lymphocytes; but usually, by comparison with known monocytes or lymphocytes, it will be seen that the histiocyte is larger than either with a more irregular cytoplasm, often exhibiting pseudopod-like projections. Typical histiocytes which were seen in some of the cases studied are shown in Fig. 1.

*Case 2 was referred to us by the Nashua Memorial Hospital of Nashua, New Hampshire, because of the unusual cells in the blood film which were observed by the technologist, Mildred Felker.

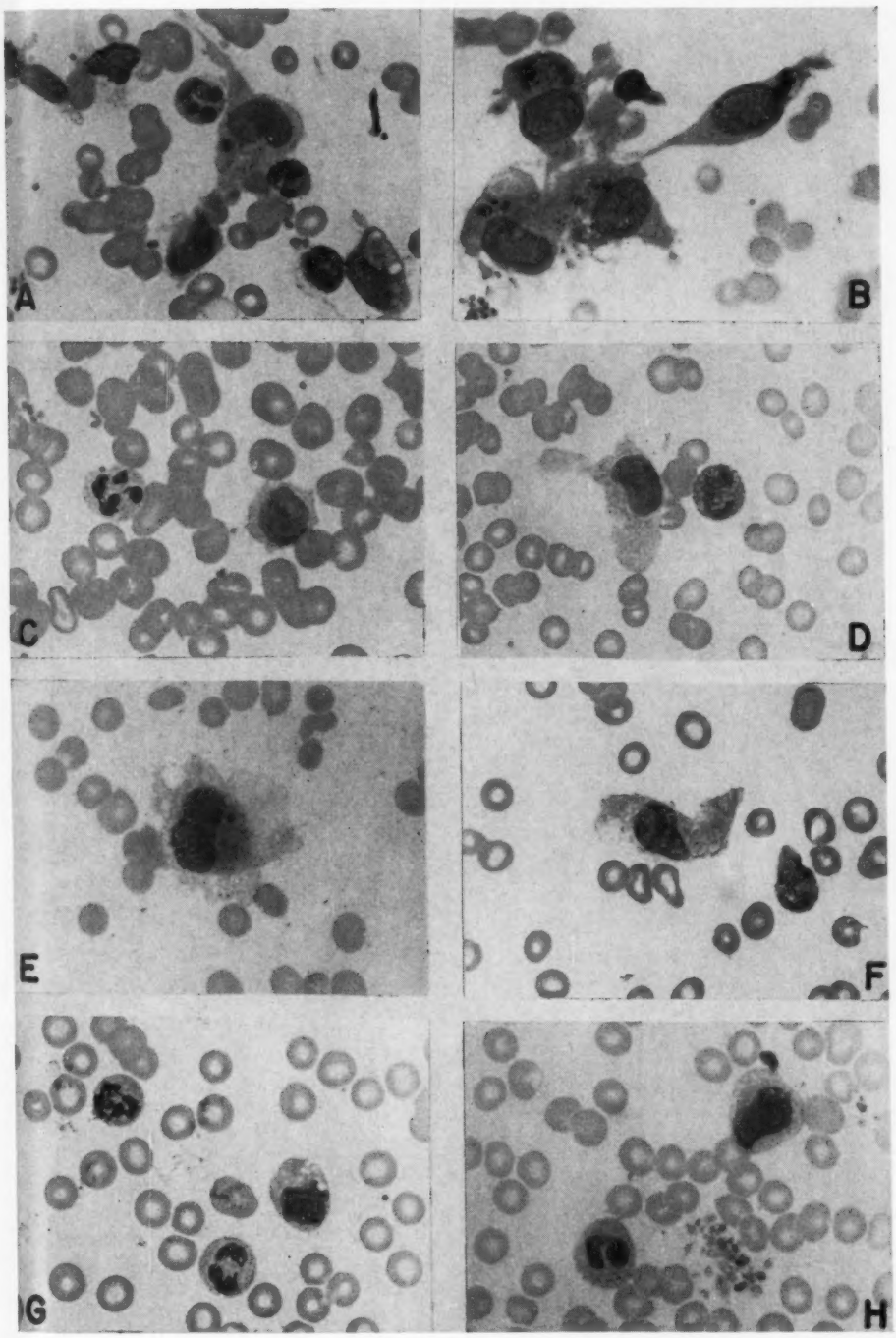


Fig. 1.—Photomicrographs showing typical histiocytes observed in the blood of patients with bacterial endocarditis. (Magnification ×800; reduced ⅙). *A* and *B* are from Case 1; *C*, from Case 3; *D*, from Case 5; *E*, from Case 6; *F*, from Case 7; *G*, from Case 8; and *H*, from Case 9.

TABLE I. SUMMARY OF CLINICAL AND PATHOLOGIC OBSERVATIONS IN TEN PATIENTS WITH BACTERIAL ENDOCARDITIS

		CLINICAL							PATHOLOGIC					
CASE NUMBER	AUTOPSY NUMBER	AGE	DIAGNOSIS	HEART	SPLEEN ENLARGED	EMBOLI	BLOOD CULTURE	DURATION (DAYS)	HEART (WT. IN GM.)	SPLEEN (WT. IN GM.)	EMBOLI	VALVE	ORGANISM	FINAL ANATOMIC DIAGNOSIS
1	A 51-534	63	? SBE ? Leukemia	Apical systolic murmur	Yes	Skin	17 negative	160	470	360	Skin	MV	Enterococcus	SBE of MV RHD with mitral stenosis
2	U 3741	61	? SBE	Aortic diastolic and systolic murmurs and apical diastolic	No	Skin	Many negative	53	360	680	Skin	AV	Alpha hemolytic Streptococcus	SBE of AV RHD with aortic stenosis
3	A 50 479	49	? SBE		No	Brain, skin	Hemolytic *Staphylococcus aureus*	28	360	290	Skin, kidney, spleen	MV	Alpha hemolytic Streptococcus	ABE of MV RHD with mitral stenosis
4	A 50 225	59	RHD SBE	Murmur	No	0	7 Negative	48	410	140	Brain, kidney	AV	Contaminant	SBE of AV Aortic calcific stenosis
5	A 43 15	70	? TB	0	Yes	0		70	320	900	Spleen, kidney, brain	AV	Pneumococcus type XI	SBE
6	A 42 61	67	ASHD with congestive failure		No			11	540	260	Brain	AV	Group D Streptococcus Enterococcus	SBE of AV ASHD
7	A 46 71	45	? SBE ? Ca	Mitral murmur	Yes	Brain	Negative	14	420	560	Spleen Right common iliac artery	MV	Alpha hemolytic Streptococcus	ABE of MV RHD

8	A	51	756	42	Pneumococcal meningitis	0	No	0	Pneumococcal type A	13	500	180	0	AV	Hemolytic *Staphylococcus aureus*	SBE of AV Bicuspid AV
9	A	46	127	58	Pneumococcal meningitis with SBE	Aortic diastolic	No	0	Negative	65	400		Kidney	AV MV	Alpha hemolytic Streptococcus	SBE of AV & MV with rupture of aortic cusps RHD
10	A	52	574	72	SBE	0	Yes	Brain	Alpha Streptococcus	5	340	290	Skin, brain, lungs, bowels, spleen, kidney	AV MV	None found	SBE of AV & MV RHD

Abbreviations:

SBE = subacute bacterial endocarditis.
ABE = acute bacterial endocarditis.
ASHD = arteriosclerotic heart disease.
RHD = rheumatic heart disease.
AV = aortic valve.
MV = mitral valve.

OBSERVATIONS

The clinical and pathologic data are summarized in Table I.

At post-mortem examination, 6 of the patients, Cases 1, 2, 3, 7, 9, and 10, were found to have had a pre-existing rheumatic lesion and 1 patient, Case 8, had a congenital deformity of the aortic valve. In the other 3 patients, Cases 4, 5, and 6, there was no gross or histologic evidence of a previous valvular lesion. The bacteria cultured at post-mortem examination included a variety of organisms, among which were enterococci, alpha hemolytic streptococci, hemolytic staphylococci, and pneumococci. The spleen was found to be enlarged in 8 of 9 cases in which the size was recorded. Embolic phenomena were present in 9 of the 10 cases.

The presence of histiocytes in the peripheral blood and the variations in white blood cell counts from the ear in Case 1 stimulated extensive hematologic studies of these phenomena. This patient remained a diagnostic problem until post-mortem examination. White cell counts and blood films for differential counts were made at frequent intervals from the ear, finger, or vein on certain days and daily on successive days in Cases 1 and 2. Illustrative results from these, as well as from the other 8 patients with subacute bacterial endocarditis, are presented in Table II. The range of the white cell counts in blood samples from the various sources in different patients was great, the lowest value being 2,800 per cubic millimeter in Case 6 and the highest 152,000 in Case 1, both being samples from the ear.

Examples of the striking differences between simultaneous white cell counts from the ear, finger, and vein in Case 1 are shown in Table II. All such data obtained on this patient are plotted in Fig. 2. Statistical analysis of these differences, based on all the data obtained, is given in Tables III and IV. The average of the white cell counts taken from the ear lobe was greater than that of those taken from either the finger tip or antecubital vein by a ratio of 7:1. In only 1 of many instances when simultaneously performed were the individual values close. Similar though much less striking differences in white cell counts between ear lobe and finger tip or vein are evident in the averaged white cell counts on the normal individuals included in Tables III and IV. These counts give an average ratio of ear counts to vein counts of 1.3:1. However, in every instance the white cell count from the ear exceeded that from the vein in the normal subjects. No significant differences were found between simultaneous white cell counts made on blood from the finger and from the vein in either Case 1 or in the normal individuals.

The differences between the total number of white cells in the blood from the ear lobe and from the antecubital vein of Case 1 were the result of increases of all types of white cells. However, these increases were quantitatively different with respect to each type of white cell. Thus, the average ratio of the number of histiocytes in the blood from the ear lobe to that from the vein was 76:1, whereas such ratios for granulocytes, lymphocytes, and monocytes were, respectively, 7.4:1, 7.8:1, and 9.3:1, as may be seen in Table IV. In 10 normal individuals these average ratios for granulocytes, lymphocytes, and monocytes

were, respectively, 1.3:1, 1.2:1, and 1.6:1. It was striking that even in the normal subjects, in only 2 instances did the number of cells of a given type in the venous blood exceed that in the blood from the ear. These exceptions were for lymphocytes. The mean values for the diameter of 100 white cells of each type obtained from the ear lobe blood of Case 1 are given in Table IV. As measured in stained blood films, the average of 2 diameters of 100 histiocytes in this series was 19.7 μ. The irregular shape was striking; among these histiocytes were individual cells whose least and greatest diameters measured, for example, 18.5 x 46.5, 20 x 31, 23 x 26, and 26 x 34 μ, respectively.

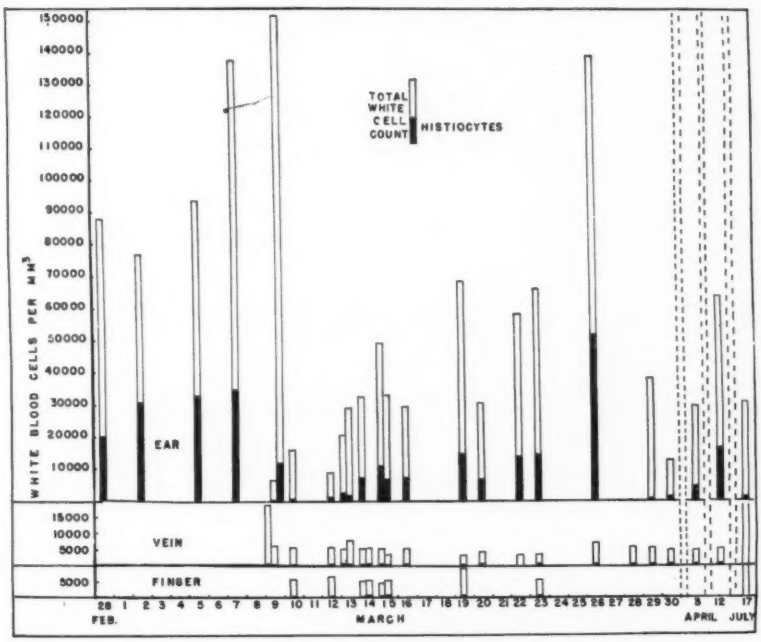


Fig. 2.—Graph showing the white cell counts of blood samples simultaneously obtained from the ear lobe, finger tip, and antecubital vein of Case 1.

The white cell count in successive drops from the ear of Case 1 varied more than could possibly result from technical errors in counting. In about one-half of the observations (6 out of 13) the white cell count was greatest in the first drop obtained after needle puncture. The proportion of histiocytes also varied in successive drops, sometimes being greatest in the second or third drop. The striking differences in the numbers of leukocytes in the blood films made from the ear lobe at intervals of a few hours in Case 1 may be seen in Table II and in Fig. 3. The day-to-day variations in white cell counts during the whole period of observation of Case 1 are shown in Fig. 2 with illustrative, daily, single, or multiple counts recorded in Table II. It can be seen that these fluctuations were much greater in blood taken from the ear lobe than in blood taken from either the finger or the vein. This variation in white cell counts in blood from the ear lobe was distinctly greater than in 4 normal individuals

TABLE II. REPRESENTATIVE WHITE BLOOD CELL VALUES OF PATIENTS WITH BACTERIAL ENDOCARDITIS

CASE NO.	SEX	AGE	DATE	TIME	WHITE BLOOD CELLS × 10^3/MM^3			NEUTROPHILS		EOSINO-PHILS (%)	BASO-PHILS (%)	MYELO-CYTES (%)	LYMPHO-CYTES (%)	MONO-CYTES (%)	HISTIO-CYTES (%)	PLASMA CELLS (%)
					EAR	FINGER	VEIN	ADULT (%)	YOUNG (%)							
1	M	63	Feb. 26, 1951	A.M.			6.8	22	57	1		1	11	6	1	1
			Feb. 28, 1951	A.M.	88.0			21	36				17	2	23	1
			Mar. 2, 1951	A.M.	77.0			19	20	1			15	4	41	
			Mar. 5, 1951	A.M.	94.0			14	25	1			20	4	36	
			Mar. 7, 1951	A.M.	138.0			24	28	1			16	5	26	
			Mar. 9, 1951	10:00 A.M.	32.0			22	22		1		21	5	29	
							19.0	39	39	1			13	6	1	1
				11:15 A.M.	6.2			30	47				13	3	7	
							6.0	37	38	2			17	4	2	
				3:30 P.M.	152.0			38	32	1			13	8	8	
			Mar. 14, 1951	10:15 A.M.	32.3			21	19	1		1	29	5	24	
						4.8		29	28	2	1		26	8	6	
							5.1	22	43				25	8	2	
				2:45 P.M.	10.2											
						5.1		32	42	1		1	15	7	2	
							4.9	30	37				24	5	3	
			Mar. 15, 1951	9:45 A.M.	49.6			18	24	1	1		28	6	22	
						4.5		27	37				26	7	2	1
							5.1	29	30	1	1		27	7	5	
				4:45 P.M.	33.6			27	19	1		1	27	5	20	
						5.2		32	28	1	1	1	25	8	4	
							4.3	35	36	2		1	20	5	1	
			Mar. 16, 1951	11:00 P.M.	29.2			19	14	1	1		33	7	25	
							4.9	25	36	1	1		28	5	4	
			Mar. 19, 1951	1:00 P.M.	68.7			24	27	1		4	18	5	21	
						5.6		27	33	2		1	25	10	2	
							3.8	35	30	3	1	1	21	6	2	
			Mar. 20, 1951	1:30 P.M.	30.3			21	27	2	1		23	4	22	
							4.1	27	36	2	1	1	23	8	2	
			Mar. 22, 1951	11:00 A.M.	58.3			17	21	1	1		29	7	24	
						9.0										
							3.6	23	36	1	1		30	9		
			Mar. 23, 1951	10:30 A.M.	66.1			15	17	1			36	9	22	
						5.4		28	30	2			26	12	2	
							3.4	24	35	1			31	6	3	
			July 17, 1951	A.M.	31.6			37	42			2	11	3	5	
						10.0		48	45			2	2	3		
							18.9	42	45			4	5	3		1

2	F	65	Apr. 28, 1937		20.5			19	16			5	44	10	6	
			May 12, 1937	2:00 P.M.	11.1			40	41			2	10	5	2	
				6:00 P.M.	9.0			39	28			6	9	8	10	
			May 13, 1937		9.0			30	40			2	21	5	2	
				A.M.	21.6			14	38			3	30	7	7	1
			May 19, 1937	A.M.	---			19	36			6	27	8	4	
				6:30 A.M.		12.9		20	28			5	36	11		
			May 20, 1937	7:00 A.M.	29.4			15	39	1		6	30	3	6	
						10.8		23	44	1		6	21	5		
3	M	49	June 2, 1950				5.4	39	49	1		1	2	7	1	
			June 12, 1950		20.0			59	27		2		6	3	3	
			June 14, 1950		17.5			70	24				3	3		
4	F	59	Feb. 3, 1950		16.0			73	7	1		1	11	5	2	
			Mar. 14, 1950		17.9			73	7				13	5	2	
5	M	70	Nov. 2, 1942		8.7			38	29	2	1		15	9	5	
			Nov. 3, 1942		---			48	20	2	1	1	15	7	6	
			Nov. 11, 1942		4.1			36	34		1		13	14	2	
6	M	67	Sept. 26, 1941		2.8			42	24	5	1		13	9	6	
			Oct. 1, 1941		6.2			46	23				19	8	4	
			Oct. 3, 1941		9.5			35	47				7	11		
			Oct. 7, 1941		5.1			28	40				14	18		
			Jan. 23, 1942		15.5			44	37	1		2	8	6	2	
7	M	45	Jan. 29, 1946				19.3	54	36				3	5	2	
			Feb. 9, 1946				22.4	46	48	1			1	3	1	
8	M	42	Oct. 10, 1951		30.7			73	19				2	4	1	1
			Oct. 12, 1951		25.0			64	21				6	7		1
9	M	58	Feb. 21, 1946		8.1			32	45	2	1		13	7		
			Feb. 27, 1946		---			31	38	1	1		20	6	4	
10	M	72	Aug. 16, 1952		---			29	55				10	5	1	
			Aug. 18, 1952				8.4	31	55			1	9	4		

Samples of blood were taken from ear lobe, finger tip, or antecubital vein.

or in 2 patients with chronic myelogenous leukemia in whom similar daily counts were made by one of the authors (G. A. D.). The statistical analysis of these results is shown in Table V.

TABLE III. STATISTICAL ANALYSIS OF THE DIFFERENCES IN TOTAL WHITE CELL COUNTS TAKEN SIMULTANEOUSLY FROM DIFFERENT SITES IN A PATIENT WITH BACTERIAL ENDOCARDITIS, CASE 1, AND ONE SET OF OBSERVATIONS IN EACH OF FIFTEEN NORMAL INDIVIDUALS

CLINICAL MATERIAL	NO. OF OBSERVATIONS	SOURCE OF SAMPLE	MEAN WHITE CELL COUNT PER MM^3	SOURCE OF SAMPLE	MEAN WHITE CELL COUNT PER MM^3	MEAN DIFFERENCE AND STANDARD ERROR OF THE MEAN PER MM^3	P VALUE
Case 1	22	Ear	36,200	Vein	5,500	30,700 ± 6,580	<0.001
Case 1	13	Ear	32,900	Finger	5,800	27,100 ± 5,800	<0.001
Case 1	12	Finger	5,830	Vein	5,814	16 ± 950	>0.1
Normals	15	Ear	8,860	Vein	6,900	1,960 ± 320	<0.001
Normals	15	Ear	8,860	Finger	7,230	1,630 ± 290	<0.001
Normals	15	Finger	7,230	Vein	6,900	330 ± 190	>0.1

Study of the white cells of the other 9 cases of bacterial endocarditis frequently showed an increase in the number of histiocytes in blood samples obtained from the ear lobe: up to 10 per cent in Case 2, and from 1 to 6 per cent in some of the other cases. The number of monocytes varied from normal values to 22 per cent, 7 observations in 4 patients yielding values above 10 per cent. Throughout the observations the neutrophils showed a definite increase in young forms. The terminal blood picture became a toxic neutrophilic

TABLE IV. COMPARISON OF AVERAGE TOTAL AND DIFFERENTIAL WHITE CELL COUNTS OF SAMPLES FROM THE EAR AND FROM THE VEINS TAKEN SIMULTANEOUSLY IN ONE PATIENT WITH BACTERIAL ENDOCARDITIS (CASE 1, 17 OBSERVATIONS) AND IN TEN NORMAL INDIVIDUALS (1 OBSERVATION EACH)

WHITE BLOOD CELLS	RATIO EAR/VEIN		MEAN CELL DIAMETER (IN MICRONS*)
	Case 1	*Normals*	
Total counts	7.0:1	1.3:1	---
Granulocytes	7.4:1	1.3:1	13.2
Lymphocytes	7.8:1	1.2:1	12.6
Monocytes	9.3:1	1.6:1	15.9
Histiocytes	76.0:1	---	19.7

*Based on the measurement of 100 cells of each type in stained blood films from Case 1.

response. This is illustrated by the last observation on Case 1 in which there were 80 to 95 per cent neutrophils with toxic granulation. The same is true of the terminal stages of Cases 3, 8, and 10. When the percentage of granulocytes was large, the percentage of histiocytes was necessarily small. However, many histiocytes were seen when the blood films were carefully inspected. Myelocytes were seen in the peripheral blood of 7 of the patients and reached 6 per cent on several occasions in the blood of Case 2. Lymphocytes, eosinophils, and basophils were within normal limits. Plasma cells were observed in the blood of 3 of the patients.

Platelets, as estimated from inspection of films of the peripheral blood, were normal or slightly decreased. Normal platelet counts were obtained on Case 1: 256,000; 197,000; 280,000; and 227,000 per cubic millimeter.

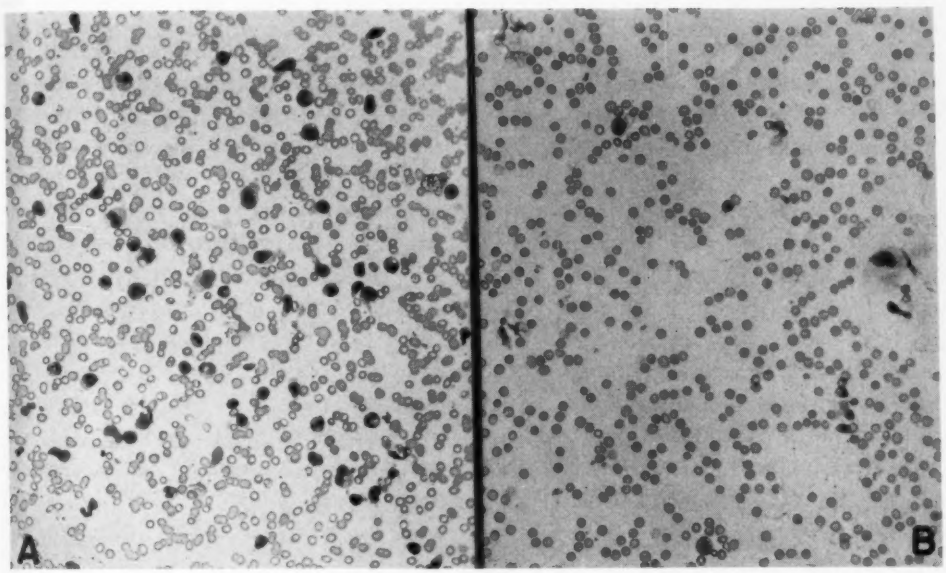


Fig. 3.—Photomicrographs of blood films taken from the ear lobe of Case 1 at *A*, 10 A.M. and *B*, at 11:15 A.M. on March 9, 1951. (Magnification ×250; reduced ¼.) *A*, White cell count = 32,000 per cubic millimeter; histiocytes = 29 per cent. *B*, White cell count = 6,000 per cubic millimeter; histiocytes = 7 per cent.

Bone marrow aspirations were not of diagnostic value and showed no increase of histiocytes or reticulum cells. In Case 1 the biopsy as well as the aspiration of the bone marrow showed granulocytic hyperplasia. Aspiration of the bone marrow of Case 4 showed about an equal proportion of erythroid and myeloid elements, while in Case 10 there was hyperplasia of the granulocyte precursors.

TABLE V. DAILY VARIATION OF WHITE CELL COUNTS IN DIFFERENT CONDITIONS

MATERIAL	SOURCE OF SAMPLE	NO. OF OBSERVATIONS	MEAN AND STANDARD DEVIATION OF MEAN	COEFFICIENT OF VARIATION (PER CENT)
Bacterial endocarditis				
Case 1	Ear,	66	31,000 ± 35,000	± 110.5
	Finger	25	5,700 ± 2,000	± 36.1
	Vein	19	5,700 ± 2,900	± 51.5
Normal individuals				
1	Ear	25	8,500 ± 1,800	± 20.9
2	Ear	35	8,600 ± 2,400	± 28.6
3	Ear	19	8,800 ± 1,300	± 14.9
4	Ear	25	6,700 ± 2,500	± 39.8
Myelogenous leukemia				
Case 1	Ear	22	63,300 ± 11,600	± 18.3
Case 2	Ear	22	109,200 ± 12,100	± 11.1

Red cell and hemoglobin values in 9 of the 10 patients with bacterial endocarditis are presented in Table VI. Anemia of moderate degree was present in all and increased as the disease progressed. The red cells were either normocytic or macrocytic, and only slightly hypochromic. In 2 of the patients, Cases 2 and 4, the reticulocytes were somewhat elevated, the highest values

TABLE VI. RED CELL VALUES IN NINE CASES OF BACTERIAL ENDOCARDITIS

CASE NO.	DATE	RED BLOOD CELLS (10^6/MM^3)	HEMOGLOBIN (GM.)	M. C. V. (μ^3)	M. C. H. C. (PER CENT)	RETICULOCYTES (PER CENT)	NUCLEATED RED CELLS (PER 100 WHITE CELLS)	ICTERUS INDEX (UNITS)
1	Feb. 26, 1951	3.8	10.9			0.3		5
	Mar. 10, 1951	3.5	9.0	79	32	1.0		4
	Mar. 15, 1951						0.5	
	Mar. 27, 1951	3.0	7.2					
	July 17, 1951	2.1	7.7	100	35			
2	Apr. 28, 1937	3.1	10.1					15
	May 12, 1937	2.9	9.2					
	May 14, 1937	2.8	9.5	121	28			
	May 15, 1937					5.7		
	May 16, 1937					5.5		
	May 18, 1937					4.9		
	May 19, 1937					4.6		
	May 20, 1937					4.9		
3	June 1, 1950	3.9	12.8	107	30	0.4		5
4	Feb. 3, 1950	2.7	9.0			9.5	1.5	10
	Feb. 28, 1950	3.1	8.8	102	28	7.1	0.5	
	Mar. 7, 1950	3.6	10.0					
5	Nov. 2, 1950					2.1		
	Nov. 3, 1950	3.7						
6	Sept. 26, 1941	2.8	8.9	92	35			5
	Oct. 7, 1941	3.3	8.9	86	32			5
	Oct. 14, 1941	2.9	9.0	95	33			4
	Jan. 23, 1942	3.7	10.4	84	34	2.5		5
7	Jan. 29, 1946	2.6	6.5	80	32			20
	Feb. 9, 1946	3.4	7.8	76	30		1.0	15
8	Oct. 12, 1951	2.6	8.5					
10	Aug. 8, 1952	3.2	8.0			2.9		5

M. C. V. = mean corpuscular volume (cubic microns).
M. C. H. C. = mean corpuscular hemoglobin concentration (grams per 100 ml. cells).

being 5.7 and 9.5 per cent, respectively. Nucleated red cells were seen in the peripheral blood films of at least 4 of the patients. Stippled red cells were occasionally observed. Spherocytes were sometimes noted in films of peripheral blood, but the osmotic fragility of the red cells was normal in the 4 patients on whom the test was carried out.

DISCUSSION

The presence of histiocytes in the peripheral blood of patients with bacterial endocarditis has been reported many times in the literature. The first accounts were by Van Nuys,[2] Bartlett,[3] and Rowley[4] and indicated that at times large numbers of these cells were present in the blood samples examined. Moreover,

their number varied remarkably from day to day and from hour to hour, sometimes even in successive drops of blood, as did the total white cell count. In Bartlett's, later Rowley's, patient the total white cell levels were sufficiently elevated to suggest leukemia: 700,000 per cubic millimeter near the end. Initially Bartlett found that there were marked variations in total (30,000 to 300,000 per cubic millimeter) and differential white cell counts in blood from the ear lobe but that in both respects blood samples from the fingers and toes remained relatively constant and within the normal range. Many of the histiocytes seen in the fixed smears were engaged in phagocytosis; and according to Rowley this activity was prominent and progressive in fresh warm-stage preparations where it was participated in by all types of white cells. Thus, from these first descriptions, the essential characteristics of the phenomenon of particular interest to us here were clearly defined, although the 2 patients were not autopsied and endocarditis was apparently not suspected despite signs of free aortic regurgitation and clubbing of the fingers in both.

Subsequent reports generally confirm these salient features as characteristic in many instances of proved subacute bacterial endocarditis. However, histiocytes displaying active phagocytosis have been reported in other conditions: typhoid fever, parasitic diseases, acquired hemolytic anemia, sickle-cell anemia, pernicious anemia, transfusion reactions, erythroblastosis fetalis, and during overwhelming infection. There is, however, general agreement that if such conditions as well as leukemia are excluded, the finding of large numbers of these cells is highly suggestive of subacute bacterial endocarditis. Thus, histiocytes (or large mononuclear or endothelial cells) were noted by many subsequent authors to be present especially in blood smears made from the ear lobe. In the European literature this finding became known as the Bittorf[6] phenomenon following this author's report in 1920. Schilling[5] reported 2 cases with 35.8 and 52.7 per cent of "monocytes," respectively, in the blood. Joseph[10] reported 7 cases in which histiocytes made up from 20 to 80 per cent of the white cells in blood drawn from the ear lobe. Sampson and his associates[8] reported 2 cases with marked fluctuations in both the white cell count and the percentage of histiocytes in blood samples, from unnamed sites. In 1 patient these abnormal cells reached 48 per cent. In the other, 4 hours after an observed level of 38, the proportion of histiocytes was only 1 per cent. Sampson concluded that these fluctuations had no relation to temperature, respiration, or pulse rate and noted the unusual size of the cells—some being 80 μ in diameter. Pepper[13] reported from 10 to 26 per cent of "monocytes" in the ear lobe blood of a few patients in whom these cells were especially looked for. Some of these cells were vacuolated and phagocytic. In 1 of these patients, many such cells were found in blood from the ear lobe but very few in blood from the finger tip. Repeated examinations for histiocytes were eventually successful in all of 10 patients with subacute bacterial endocarditis studied by Ottander.[11] In a recent series of 122 patients reported from the Mayo Clinic by Parsons and his associates,[15] in only a small number of whom was the first drop of blood from the ear lobe examined, "endothelial" cells were found in 37 with evidence of phagocytosis in 11.

Others, notably Bittorf[6] and Hess,[7] have reported that histiocytes may be especially numerous in the first drop of blood obtained from the ear lobe. Authorities disagree as to whether avoidance of preliminary manipulation or on the other hand massage of the ear lobe increases the number of such cells in the preparation.[12] In our series of 10 patients, either the first or second drop of blood was used without special manipulation of the ear lobe. Histiocytes were found at some time in the blood of every patient. The highest proportion of these cells was 41 per cent in blood obtained from the ear lobe of Case 1. No correlation was found between variation in the white cell counts or presence of histiocytes and clinical manifestations such as fever, signs of infarct, of emboli, or of cardiac failure. In our patients there was almost always some relative increase in young neutrophils in the blood samples obtained from any site.

Many authors have reported fluctuating numbers of monocytes in the blood of patients with bacterial endocarditis, but the results are hard to evaluate because this term is often used to include histiocytes. Indeed, Schilling[5] reported what he interpreted as a continuous series of cell types ranging from typical large mononuclears to atypical mononuclear cells with voluminous cytoplasm as well as spindle-shaped endothelial elements. Ingested white cells, red cells, and portions of cells, as well as empty vacuoles, are frequently mentioned or illustrated,[4, 5, 11-14, 16] as characteristic of phagocytic mononuclear cells or histiocytes in endocarditis. Rowley[4] found that mixture of the blood of her patient with normal blood produced marked phagocytosis by normal mononuclear cells after an hour in the warm chamber. The finding of active phagocytosis is not surprising in view of the intense hyperimmune state[19] of patients with subacute bacterial endocarditis, which is expressed by rapid destruction of organisms in vitro only when white cells are present.[20] However, as Simpson[21] has produced large phagocytic cells in the blood stream of the rabbit by injecting colloidal substances, the stimulus to their production in patients appears to be the immediate result of stimulation of the reticuloendothelial system.

Another feature of the peripheral blood of certain of these patients is the abnormally high and fluctuating total white cell counts observed in blood samples obtained from the ear lobe and almost never, where the source of the sample is recorded, from the finger tip or vein. Rowley's[4] patient appears to have shown the highest white count recorded in the literature—700,000 in blood from the right ear 5 days after a white cell count of only 25,000 and 9 days after a white cell count of 430,000 per cubic millimeter from the same source. White cell counts varying between 19,200 and 125,000 were found by Broustet and his associates[14] in blood samples from the ear, while venous white cell counts ranged only from 3,000 to 7,500 per cubic millimeter. Hess[7] reported a case with simultaneous white cell counts in blood from the ear lobes of 75,000 (right) and 112,200 (left) but from the finger tip of only 4,390 per cubic millimeter. In our patients the highest white cell count, 152,000, was found in blood from the ear lobe of Case 1 on a day when only a few hours earlier simultaneous white cell counts from the ear and vein had been 6,200 and 6,000 per cubic millimeter,

respectively (Table II). On many other occasions in Case 1 simultaneous white cell counts from the ear, finger, and vein showed that the white cell count in the ear lobe was greater than that in either the finger or the vein by five- or tenfold. Indeed, there was a tendency for the venous white cell counts to be slightly subnormal—not only in Case 1 but in occasional patients reported by others. Thus, the fact that leukocytosis may be absent in blood samples from finger or vein does not exclude chronic bacterial infection of the heart valves even with bacteremia.

By analogy with other chronic infections in which histiocytes are seen in the peripheral blood, a generalized response of the reticuloendothelial tissues presumably explains the presence of these cells in the circulation in subacute bacterial endocarditis.[1] Ottander[11] noted hyperplasia of the endothelial cells lining the capillaries and somewhat larger vessels, especially the veins in histologic preparations obtained at autopsy from the liver, spleen, and bone marrow. In addition he observed endothelial proliferation in the vessels of the renal glomeruli. Ottander also examined sections of the ear lobe and finger tip. Here, too, there were proliferation and swelling of the endothelial cells lining the blood vessels, occasionally to the point of obliteration. The proliferative process was somewhat more marked in the ear lobe than in the finger tip. Both Hess[7] and Ottander[11] concluded that the peripheral blood vessels as well as those of the internal organs released "monocytes" into the blood stream. Hess apparently thought that increased vascularity, lack of muscular contraction, and relative freedom from trauma played a part in causing the local increase in "monocytes" in the ear lobe.

Jaffé[22] considered that, in addition to local formation, macrophages (histiocytes) free in the circulating blood might become arrested by the swollen and protruding endothelial cells of the small blood vessels of the ear lobe. Although Simpson[21] had previously ascribed to the filtering action of the pulmonary capillaries of rabbits injected with colloidal dyes, the striking preponderance of histiocytes, especially the larger forms, in the blood of the right heart as compared to that of the periphery (sometimes as much as 70 compared to 6 per cent), Jaffé's statement for the first time clearly implies the concept of a selective filtering action of the ear-lobe vasculature. Such a hypothesis is obviously necessary in order to explain the fact that the significantly higher total white cell counts in blood samples obtained by several authors from the ear lobe compared to those from the finger or vein are the result in large part of increases of cells originating elsewhere in special tissues such as bone marrow and lymph nodes: for example, granulocytes and lymphocytes. Thus, Bittorf[6] obtained a white cell count of 18,050 with 57 per cent of "endothelial" cells in blood from the ear of a patient with endocarditis, while on two occasions blood from the vein contained only 2,400 and 3,100 white cells and no "endothelial" cells. Broustet and his associates[14] recorded white cell counts from the ear lobe of their patient on one day at 10 A.M., 3 P.M., and 6 P.M. of 53,900, 71,400, and 19,100 per cubic millimeter, respectively. In these samples the percentages of histiocytes were, respectively, 38, 12, and 17. By inspection of similar data from Case 1 in Table II it will be seen that the elevated white cell counts from

the ear lobe were usually associated with larger percentages of histiocytes compared to the finger or vein but that these cells by no means account for the higher white cell counts. The increased average ratios for each type of white cell in blood from the ear compared to blood from the vein of Case 1 are shown in Table IV. These differ for each kind of white cell. Whereas the average ratio of ear to vein for total white cells was 7:1, that for histiocytes was 76:1, and that for the other cell types ranged from 7.4:1 to 9.3:1.

Formation of at least some of the histiocytes in the ear lobe cannot be denied, and is indeed probable in subacute bacterial endocarditis. However, it is clear that the characteristically elevated white cell counts in the ear lobe are the result of absolute increases of other types of white cells as well as of histiocytes. At the same moment the finger or vein blood samples may show no increase or even a slight decrease in the total white cell count. Despite the speculations in the literature, these findings can be logically interpreted only as evidence of a local filtering or sequestering mechanism involving to some extent all types of white cells traversing the ear lobe. Indeed, even in normal subjects, as shown by Price-Jones and his associates[23] among others, as well as in our experience (Tables III and IV), the total number of white cells in blood from the ear lobe is usually greater than in samples from the finger or vein. Moreover, a comparatively large white cell in the blood of normal subjects, the monocyte, is relatively concentrated in the ear lobe blood as observed by Heath,[24] by Lucey,[25] and as shown in Table IV. In the present observations, without exception, the percentage of monocytes in individual samples of the ear lobe blood of 10 normal subjects exceeded that in blood samples taken from the vein at the same time. Because the concentration ratios for different types of white cells are quantitatively different, their local increases cannot be due simply to relative loss of plasma by filtration.

The filtering theory has not been seriously considered by some authors,[6, 7, 14] because corresponding increases in red cells were found not to accompany the local increases in white cells. However, it is not necessary to suppose that because a capillary system is capable of differentially restraining the passage of various types of white cells, it must necessarily do so proportionately for the far smaller red cells. However, Broustet[14] presents without comment data on 5 normal subjects showing distinctly higher values for both red and white cells in blood from the ear than in blood from the vein. Modern evidence supports the highly selective capacity of vascular filters even for different types of red cells or particles of even smaller diameter. Thus the spleen in man easily distinguishes between normal and spheroidal red cells,[26] and in the rabbit intravenously injected starch grains averaging $2.7 \pm 0.1\ \mu$ pass into the pulp while those averaging $3.51 \pm 0.02\ \mu$ in diameter remain in the sinuses.[27]

In bacterial endocarditis, the total production of histiocytes is doubtless greatly increased by the infection. Yet it is not difficult to understand that the relatively swift flow of blood in the large vessels does not allow for an accumulation of histiocytes. Moreover, the numerous arteriovenous anastomoses characteristic of the circulation of the finger tip[28] would likewise be less effective as a filter than would the slower circulation through a capillary system composed

of vessels of smaller caliber as in the ear lobe. In addition to such differences in the normal vascular anatomy, the hyperplastic changes in the vascular endothelium of the ear lobe reported[1, 11, 22] in bacterial endocarditis (as well as of the finger tip, spleen, liver, bone marrow, lungs, and glomeruli) probably help the capillaries of the ear lobe to trap the large, actively ameboid histiocytes presented by the arterial blood stream. The remarkable concentration of these cells in the ear lobe blood relative to that of other types of white cells thus probably depends principally upon their larger size with respect to the capillary bed of the ear lobe acting as a normal or even somewhat hyperplastic filter. Because in these patients large variations in white cell counts appear even in successive drops of blood from the same puncture of the ear lobe, the greater fluctuations in white cell counts from the ear lobe compared to those from the finger tip probably do not reflect so much variation in production as in local trapping or discharge of leukocytes by a filter[21, 22] normally subject to changes in temperature, dependency, and trauma.[28]

The presence of anemia in the cases recorded in this series is in agreement with many previous reports in the literature. Pepper[13] found severe anemia in all but 1 of his 20 patients with bacterial endocarditis. Recently Parsons and his associates[15] reported anemia in 75 per cent of the 200 cases they studied. They emphasized the importance of considering the diagnosis of bacterial endocarditis whenever a cardiac murmur is associated with unexplained fever or anemia. That this anemia is due in part to a hemolytic process in some patients is suggested by the elevated reticulocyte values noted in Cases 2 and 4 and the increased icterus index in Cases 2, 4, and 7 of this series.

Many laboratories customarily use blood from the finger or from the vein for cell counts and blood films. These sites probably give samples more representative of the general white cell levels in the circulation than does the ear lobe. However, when a search for abnormal white cells is desired, it is apparent that repeated and careful study of blood films taken from the ear lobe may be more useful. Thus, because of the filtering action of this tissue, the diagnosis of bacterial endocarditis or of other diseases with sparsely affected circulating leukocytes may be aided.

SUMMARY

Elevation and wide variation in white cell counts, together with numerous histiocytes, sometimes in the process of phagocytosing red cells, may be encountered in bacterial endocarditis in blood samples taken from the ear lobe and sometimes appear in successive drops of blood from this site. Such findings are much less characteristic of blood samples obtained from the finger tip or the antecubital vein.

This relative increase in the total white cell count, which involves all types of white cells, as well as the predominance of histiocytes in the blood from the ear lobe in bacterial endocarditis are probably explained largely by the greater selective filtering capacity of the vascular bed of the ear lobe compared to that

of the finger tip. Indeed, even in normal subjects the total white cell count is greater, and the monocyte, another large white cell, is statistically more common in blood samples taken from the ear lobe than from the finger tip.

The ear lobe provides a desirable site for the preparation of blood films when a search for infrequent abnormal white cells is contemplated, as in certain infections or in leukemias, especially when leukopenia is present. On the other hand, blood samples from the finger tip closely resemble those from the antecubital vein and are quantitatively more representative of the white cell content of the circulating blood.

In addition to the presence of histiocytes, the peripheral blood in bacterial endocarditis may show, especially in terminal phases of the disease, a striking granulocytosis with heavy toxic granulation of the neutrophils. The predominance of granulocytes reduces the relative percentage but not the absolute number of histiocytes.

Finally, a moderate to marked anemia, varying from normocytic to macrocytic, is usually present in bacterial endocarditis. In some patients the elevated reticulocytes and icterus index suggest that this anemia may involve a hemolytic process.

We wish to acknowledge with appreciation the technical assistance given by Phyllis Gordon Fersky and Mary Lu Hanson.

REFERENCES

1. Merklen, P., and Wolf, M.: Participation des endothéliites Artério-capillaires au Syndrome de L'endocardite Maligne Lente, Presse méd. **36:** 97-100, 1928.
2. Van Nuys, F.: An Extraordinary Blood—The Presence of Atypical Phagocytic Cells, Boston M. & S. J. **156:** 390-391, 1907.
3. Bartlett, W. B.: Localized Leucocytosis Associated With Atypical Phagocytic Cells, Boston M. & S. J. **156:** 629-630, 1907.
4. Rowley, M. W.: A Fatal Anaemia With Enormous Numbers of Circulating Phagocytes, J. Exper. Med. **10:** 78-96, 1908.
5. Schilling, V.: Ueber hochgradige Monozytosen mit Makrophagen bei Endocarditis ulcerosa und über die Herkunft der Gr. Mononukleären, ztschr. klin. Med. **88:** 377-397, 1919.
6. Bittorf, A.: Endothelien im strömenden Blute und ihre Beziehungen zu hämorrhagischer Diathese, Deutsches Arch. f. klin. Med. **133:** 64-73, 1920.
7. Hess, O.: Zur Herkunft der in strömenden Blut bei Endocarditis lenta vorkommenden Endothelien, Deutsches Arch. f. klin. Med. **138:** 330-339, 1922.
8. Sampson, J. J., Kerr, W. J., and Simpson, M. E.: A Study of Macrophages in the Human Blood With Special Reference to Their Presence in Two Cases of Subacute Bacterial Endocarditis, A. M. A. Arch. Int. Med. **31:** 830-856, 1923.
9. Seyderhelm, K.: Ueber das Vorkommen von Makrophagen im Blute bei einem Fall von Endocarditis ulcerosa, Arch. path. Anat. **243:** 462-473, 1923.
10. Joseph, F.: Hochgradige retikulo-endotheliole Monozytosen bei Endocarditis maligna, Deutsche med. Wchnschr. **51:** 863-864, 1925.
11. Ottander, O.: Hochgradige Endotheliose im Blut bei Endocarditis lenta, Acta med. scandinav. **63:** 336-354, 1925-26.
12. Fontana, L.: Richerche su di uno speciale reperto ematologica nella endocardite lenta e su reperti affini in varie altre candizione, Haematologica **7:** 271-382, 1926.
13. Pepper, O. H. P.: The Hematology of Subacute Streptococcus Viridans Endocarditis, J.A.M.A. **89:** 1377-1380, 1927.
14. Broustet, P., Saric, Léger, Bergaud, A., and Mlle. Borchard: Cellules endothélioides hémohistioblastiques et variation de la formule du sang capillaire et due sang veineux dans la maladie d'Osler, Arch. mal. coeur **39:** 294-300, 1946.
15. Parsons, W. B., Cooper, T., and Scheifley, C. H.: Anemia in Bacterial Endocarditis, J.A.M.A. **153:** 14-16, 1953.
16. Hurxthal, L. M.: Clinical Observations on Subacute Bacterial Endocarditis, Boston M. & S. J. **197:** 41-46, 1927.

17. Pohle, F. S.: The Blood Platelet Count in Relation to the Menstrual Cycle in Normal Women, Am. J. M. Sc. **197**: 40-47, 1939.
18. Ham, T. H.: A Syllabus of Laboratory Examinations in Clinical Diagnosis, Cambridge, 1950, Harvard University Press.
19. Horder, T. J.: A Case of Chronic Streptococcus Endocarditis Treated by Serum Specially Prepared by the Use of the Organism Obtained From the Patient, Lancet **2**: 143-144, 1904.
20. Friedman, M., Katz, L. N., and Howell, K.: Experimental Endocarditis Due to Streptococcus Viridans: Biologic Factors in its Development, A. M. A. Arch. Int. Med. **61**: 95-118, 1938.
21. Simpson, M. E.: The Experimental Production of Macrophages in the Circulating Blood, J. Med. Res. **43**: 77-144, 1922.
22. Jaffé, R. H.: Reticulo-endothelial System, *in* Handbook of Hematology, H. Downey, editor, New York, 1938, Paul B. Hoeber Co., Vol. II, pp. 1057-1065.
23. Price-Jones, C., Vaughan, J. M., and Goddard, H. M.: Haemotological Standards of Healthy Persons, J. Path. & Bact. **40**: 503-519, 1935.
24. Heath, C. W.: Personal communication.
25. Lucey, H. C.: Fortuitous Factors Affecting the Leucocyte Count in Blood From the Ear, J. Clin. Path. **3**: 146-151, 1950.
26. Young, L. E., Platzer, R. F., Ervin, D. M., and Izzo, M. J.: Hereditary Spherocytosis: II. Observations on the Role of the Spleen, Blood **6**: 1099-1113, 1951.
27. Björkman, S. E.: The Splenic Circulation; With Secial Reference to the Function of the Spleen Sinus Wall, Acta med. scandinav. **128**: 1-89, Supp. 191, 1947.
28. Grant, R. T., and Bland, F. F.: Observations on Arteriovenous Anastomoses in Human Skin and in the Bird's Foot With Special Reference to Cold, Heart **15**: 385-411, 1931.

STUDIES ON PLATELETS

XVII. An Experimental Study of the Development of Platelet Antibodies

Sten A. Kistner, M.D.,* and Mario Stefanini, M.D.**
Boston, Mass.

With the Technical Assistance of Rose Mele, M.T. (ASCP), and
Nancy B. Martino, A.S.

AN IMMUNOLOGIC pathogenesis has been postulated for many different hematologic syndromes involving formed elements of the blood, the vessel wall, and some plasma constituents.[1] This has been particularly emphasized in some thrombocytopenic states, where current theories stress the pathogenetic role of platelet antibodies. The origin of antibodies, however, is not clear. The present paper is an experimental study of their development and mechanism of activity in the determination of thrombocytopenia.

The thrombocytopenic syndrome has been traditionally classified in a "megakaryocytic" and an "amegakaryocytic" type (Table I). Morphologic studies of the bone marrow have represented the clue to this differentiation. The "amegakaryocytic" type of thrombocytopenia shows striking reduction in the number of megakaryocytes, usually associated with aplasia of the bone marrow or its replacement by foreign tissues (metastatic tumors, leukemias). In the "megakaryocytic" type there is a characteristic dissociation between a normal or increased number of megakaryocytes (some of which present a pathologic morphology) in the bone marrow and the peripheral thrombocytopenia. This type of thrombocytopenia has long represented a pathogenetic problem. In some instances, definite pathogenetic factors have been found. Thus, the spleen may be the seat of a definite pathologic entity, such as lupus erythematosus disseminatus, sarcoidosis, tuberculosis, lymphoma, histoplasmosis, and others. Most often in these cases the spleen is enlarged or overactive, and splenectomy is followed by complete recovery. In other instances, like in the acute purpura which follows administration of drugs like Sedormid (allyl-isopropyl-acetyl-carbamide), quinine, quinidine, aminopyrine in sensitized individuals, thrombocytopenia is presumably due to circulating agents able to destroy platelets in vivo or in vitro, but only in the presence of the offending

From the Joseph Stanton Memorial Laboratories, Saint Elizabeth's Hospital and the Department of Medicine, Tufts University School of Medicine, Boston, Mass.

Supported by Grants H-2131 and H-2132 from the National Institutes of Health, United States Public Health Service.

Received for publication April 18, 1956.

*Research Associate, Saint Elizabeth's Hospital; under a fellowship of the American-Scandinavian Foundation. Present address: Department of Medicine, Kazolinska Institute, Stockholm, Sweden.

**Director, Joseph Stanton Memorial Research Laboratories and Hematologist, Saint Elizabeth's Hospital; Associate Professor of Medicine, Tufts University School of Medicine and Established Investigator, American Heart Association.

drug. These agents are very likely antibodies,* although the stimulating antigen or antigens and the manner with which the antibody develops remain a matter of speculation. In a third type of megakaryocytic thrombocytopenia, the lack of recognizable pathogenetic mechanisms justifies the definition of "idiopathic." In these cases, the etiology of the disease has long been centered on the spleen and its function, primarily because of the often striking therapeutic results of splenectomy. The mechanism, however, through which the spleen induces thromobcytopenia has never been finally assessed. Both physical and humoral splenic mechanisms have been postulated. Physical mechanisms include direct sequestration of platelets by the spleen[2, 3] or removal from the circulation of platelets which have been previously damaged by circulating agglutinins (perhaps antibodies[4, 5]). The humoral activity of the spleen may include the total or partial production of platelet antibodies[6] or the delivery of factors capable of inhibiting the development and the maturation of megakaryocytes as well as their platelet production.[7, 8]

TABLE I. ETIOPATHOGENETIC CLASSIFICATION OF THROMBOCYTOPENIC STATES IN ADULTS

a. Amegakaryocytic	bone marrow aplasia bone marrow replacement by tumor	
b. Megakaryocytic	1. Hypersplenic	
	2. Drug-induced	
	3. Idiopathic, due to	I. decreased platelet production: a. inhibition of megakaryocyte development and function by splenic humoral factors b. inhibition of megakaryocyte development and function by circulating antibodies
		II. increased platelet destruction: a. sequestration of normal platelets in spleen b. direct attack of platelets by circulating antibodies c. sequestration in spleen of platelets damaged by antibodies

There is no question that the role of immunologic phenomena in the pathogenesis of idiopathic thrombocytopenic purpura is increasingly emphasized. Platelet agglutinins have been demonstrated in a good number of patients with the disease. The serum of these patients, prior to any transfusion, clumps the patient's own platelets as well as normal platelets in vitro; it also causes thrombocytopenia when injected in normal individuals.[9] If the patient's serum contains auto- as well as isoantibodies, this, in turn, suggests immunization against platelets. Perhaps autoimmunization is responsible for the patient's thrombocytopenia.

In investigating the pathogenetic mechanism of idiopathic thrombocytopenic purpura, one is impressed by the frequent incidence among these patients

*In the course of this work, the definition of antibody is applied to all substances capable of reacting specifically with a recognizable antigen.

(especially when suffering from the acute form of the disease) of preceding viral or bacterial infection, or of administration of drugs like aspirin, antihistaminics, etc. If the disease is due to the development of an antibody against the individual's own platelets (autoimmunization), the role of these factors requires consideration. It has been said by many that platelets, when modified* by bacterial agents or drugs, may become foreign to their own body and, therefore, possibly antigenic. Unfortunately, this theory has been entirely speculative thus far. No experimental evidence is yet available that modified platelets represent the antigen for the development of platelet iso- and autoantibodies.

The present study endeavors to offer an experimental approach to these problems. Antigenicity of platelets was studied after modification with drugs, bacterial, and viral agents. Quinine, quinidine, and aminopyrine are well known to produce thrombocytopenia in sensitized individuals. They were, therefore, employed in these experiments as most likely able to modify the antigenicity of normal platelets. *Staphylococcus albus, Staphylococcus aureaus* and *Streptococcus hemolyticus* figure predominantly as agents of infections preceding the onset of thrombocytopenic purpura. Filtrates of these organisms were therefore selected to modify platelets. Newcastle disease virus was selected because of the favorable results reported by other investigators in securing autoimmunization against red cells in animals by the employment of this virus.[10]

MATERIALS AND METHODS

Male albino rabbits, with the average weight of 6 pounds, were used in these experiments. They were fed a diet of Purina food, lettuce, and carrots at will. The rabbit was selected because of the impossibility of carrying out such a number of experiments in larger animals. Sufficient volume of blood can be collected from rabbits for abundant platelet harvesting and full-scale antibody studies. A total of 56 animals were used in these experiments, divided as follows: heteroimmunization experiments, 8; isoimmunization experiments, 28; autoimmunization experiments, 20.

1. *Preparation of Platelets.*—When not otherwise indicated, all steps involving handling of platelets were carried out with use of silicone-coated glassware and Arquad 2-C-coated needles.

a. *Human platelets:* Twenty milliliters of human O Rh + blood was collected from an antecubital vein following the 2-syringe technique.[11] One-tenth volume of 1 per cent Sequestrene-Na_2 solution† was added. Blood was centrifuged at 500 to 800 r.p.m. at 4° C. for 30 minutes. The supernatant plasma was transferred to another tube and centrifuged twice more at the same speed for 5 minutes in order that a maximum number of red cells might be separated. The red cell-free, platelet-rich plasma thus obtained was centrifuged at 1,800 r.p.m at 4° C. for 30 minutes or longer if needed, in order that all platelets in the bottom of the tube might be collected. After the discarding of the supernatant plasma, test tubes were inverted and their walls dried with filter paper. Platelets were resuspended by gentle shaking in a volume of saline solution equal to one-half of the original volume of blood. Occasional platelet clumps were separated by slow speed centrifugation (400 r.p.m. at 4° C. for 5 minutes). Supernatant was transferred to another test tube and once again spun at

*"Modification," in the course of this paper, indicates the change in antigenic properties which seems to follow treatment of platelet suspensions by various viral, bacterial agents and drugs.

†Containing 1 Gm. of Sequestrene-Na_2 and 0.7 Gm. NaCl per 100 ml.

high speed. Platelets which collected to the bottom were then washed once and finally resuspended in saline solution. Only suspensions were used which appeared free of clumps under phase microscope.

b. *Rabbit platelets:* Ten milliliters of blood was collected through an ordinary 20-gauge needle from the central artery of the ear, into a syringe containing 2 ml. of 1 per cent Sequestrene-Na_2 solution. Platelets were prepared as described for human platelets, but no final washing was done, since this was often followed by irreversible clumping.

Most preparations of human as well as rabbit platelets averaged 300,000 per cubic millimeter in number.

2. *Modification of Platelets.*—Modification of platelets was attempted with four different techniques: (a) washing; (b) incubation with drugs; (c) incubation with bacterial filtrates; (d) incubation with viruses.

a. *Washing:* Platelet suspensions in saline, prepared as described from either 20 ml. of human blood or 10 ml. of rabbit blood were left at room temperature for one hour. Platelets were then washed 3 times and resuspended in 2 ml. of freshly prepared saline solution.

b. *Incubation with drugs:* The maximum concentration of each drug not causing agglutination of platelets was first determined in preliminary experiments. Serial dilutions in saline were prepared from stock 1 per cent solutions of aminopyrine, quinine hydrochloride, and quinidine sulfate. To 0.1 ml. of each dilution of the series was added 0.1 ml. platelet-rich plasma; mixture was incubated at room temperature for one hour, then centrifuged at slow speed for 2 minutes. Presence of agglutination was checked by phase microscopy under 460× magnification. Used for platelet modification was the highest concentration of drug not causing their clumping (Table II). Equal volumes of a solution of the drug of chosen concentration and platelet-rich plasma or platelet suspension from 20 ml. of human or 10 ml. of rabbit blood were incubated at room temperature for one hour. Platelets were then separated by centrifugation, washed 3 times, and resuspended in 2 ml. of saline solution.

TABLE II. HIGHEST CONCENTRATION OF VARIOUS DRUGS NOT CAUSING AGGLUTINATION OF PLATELETS WHEN ADDED TO PLATELET-RICH HUMAN AND RABBIT PLASMA

	AMINOPYRINE	QUININE-HCL	QUINIDINE SULFATE
Human platelets	1.25 mg./ml.	2.5 mg./ml.	1.25 mg./ml.
Rabbit platelets	2.50 mg./ml.	0.078 mg./ml.	0.078 mg./ml.

c. *Incubation with bacterial filtrates:* Cultures of virulent ß-hemolytic Streptococcus and *Staphylococcus aureus* and *albus* from human sources (throat culture) were inoculated in 10 ml. of broth medium (Bacto beef extract, Difco) at 37° C. for 24 hours. Cultures were then filtered through Seitz. Collected filtrates contained from 385 to 460 mg. per cent nitrogen. As they did not appear to cause clumping, modification of platelets was carried out by incubating equal volumes of filtrate and platelet-rich plasma or suspension of platelets from 20 ml. of human or 10 ml. of rabbit blood. Further steps were as indicated in "b." Filtered sterile broth was used to modify platelets in control studies.

d. *Incubation with Virus:* Newcastle disease virus, B-1 strain, growing in allantoic fluid, with a hemagglutinin titer of 1/320 and egg infectivity titer of $10^{-8.5}$ was used. Sterile allantoic fluid of the same age was used as control.* For modification of platelets, equal volumes of virus suspensions diluted 1:40 with saline or allantoic fluid diluted 1:16 (highest concentrations not causing agglutination of platelets) and platelet-rich plasma or platelet suspensions from 20 ml. of human or 10 ml. of rabbit blood were incubated and further treated as in "b."

3. *Immunization of rabbits.*—Three different kinds of modified platelets were used for immunization of rabbits. One group received human (heterologous) platelets, another group

*Courtesy of Dr. Henry Van Roekel and Mr. Edward H. Seadale, Department of Veterinarian Science, University of Massachusetts, Amherst; and Dr. Alvin Levine, Department of Bacteriology, Harvard Medical School, Boston, Mass.

received platelets from normal rabbit donors (isoplatelets), and a third group received their own platelets (autoplatelets). Modified platelets were injected in the marginal vein of the ear in a volume of 2 ml. Control rabbits were immunized with direct injection of 2 ml. of 1:40, 1:20, or 1:10 suspension of Newcastle disease virus in saline. Other controls received quinidine 7.5 mg. in 0.75 ml. of saline or 1 ml. of 1:1 bacterial filtrates. All animals received 3 injections with one-week interval between them. Ten milliliter of blood was collected from the central auricular artery prior to each new injection and 4 to 7 days after last administration for antibody studies. A fresh blood sample was also collected from some rabbits 3 months after the last immunizing injection. In each instance serum was obtained by incubating blood at 37° C. for one hour and promptly separating the serum by centrifugation. Serum was then divided in 1 ml. aliquots, frozen, and stored at −20° C. Plasma rather than serum was collected from animals being autoimmunized, to prevent repeated bleeding within a few hours.

4. *Counting of Platelets.*—Blood was collected from the central artery of the ear through a 25-gauge needle to the mark of 1 ml. in a nonsiliconized tuberculin syringe containing 0.4 ml. 1 per cent Sequestrene-Na_2 solution. Blood was collected in the syringe without aspiration, then transferred to a siliconized glass test tube. Platelets were counted with the procedure previously described.[12] Wide variations were found in *different* animals. In the same control animal, however, range of variations averaged ± 12.5 per cent. In the analysis of results, only figures indicating variation in the platelet count of the same experimental animal of more than 25 per cent were considered significant. The same method was applied when counting platelets suspended in saline or in plasma.

5. *Detection of Agglutinating Antibodies in Vitro.*—Each serum tested for agglutinating activity was first inactivated at 56° C. for 30 minutes, then diluted serially with saline solution. One-tenth milliliter of each dilution was thoroughly mixed with 0.1 ml. of platelet suspension in saline. Mixtures were incubated at room temperature for one hour. Test tubes were then centrifuged at slow speed for two minutes. After gentle shaking their content was poured over silicone-coated glass slides* and spread by applying glass cover slips. Presence or absence of agglutination was determined by direct reading with phase microscope at 460× magnification. A single platelet suspension was used to test sera collected at different times from each rabbit. Rabbit sera tested against human platelets were first treated with ion exchange resin Dowex-50† to prevent aspecific agglutination of platelets and even clotting of the suspension. Modified and control platelets were tested in the presence and in the absence of the modifying agent.

6. *Antibody Absorption Experiments.*—Sera from rabbits showing detectable platelet agglutins were absorbed with normal as well as modified platelets by previously published techniques.[13] After absorption, sera were retested against the platelets they agglutinated originally.

7. *"Incubation Experiments" in vitro.*—Blood samples were collected from immunized rabbits as described in paragraph 4. Samples of blood were also collected in one-fifth volume of saline solution containing 100 γ per milliliter heparin sodium. Specific as well as nonspecific modifying agents were added to the blood samples in the same concentration at which they were used to modify platelets. Platelet counts were taken immediately and after two hours of incubation at 37° C. Identical experiments were performed with use of blood from normal rather than from immunized rabbits.

8. *Complement-Fixation Test.*—A modification of Kolmer test[14] was used. Fixing antibodies were studied in rabbit sera prior to and following immunization. The antigen was represented by standard suspensions of platelets prepared from either normal rabbits or

*Slides were treated with 20 per cent solution of silicone (SC 887) in petroleum ether, repeatedly washed with distilled water, and dried in oven at 75° C. for 2 hours. They were stored in alcohol 70 per cent. At the time of use they were washed with distilled water and dried manually with lint-free gauze.

†1 Gm. of resin was used for each 10 ml. of serum.

from human O, Rh + individuals. Lyophilized commercial guinea pig complement was used.* Complement was titrated against normal platelet suspensions before each test for determination of anticomplementary level.

9. *Injection of Modifying Agents in Immunized Animals.*—Each immunized rabbit received intravenously the modifying agent specifically used, one to two weeks after the last immunizing injection. Doses used are shown in Table III. Two milliliters of saline solution was injected in the control animal. Platelet counts were taken immediately before, 5, 15, 30 minutes, and 2 and sometimes 24 hours following the injection. In control experiments, normal rabbits received modifying agents, and immunized rabbits received agents to which they had not been exposed. In some instances these experiments were repeated 3 months after the last immunizing injection.

TABLE III. DOSES OF MODIFYING AGENTS INJECTED INTO IMMUNIZED RABBITS

	VOLUME	CONCENTRATION*	AMOUNT
a. Drugs			
Aminopyrine	2 ml.	1%	20 mg.
Quinine	0.75 ml.	1%	7.5 mg.
Quinidine	0.75 ml.	1%	7.5 mg.
	VOLUME		DILUTION*
b. Bacterial filtrates	1 ml.		1/1
Broth (sterile)	1 ml.		1/1
c. Newcastle disease virus suspension	2 ml.		1/20
Allantoic fluid	2 ml.		1/20

See Material and Methods, paragraph 9.
*In saline solution.

10. *Injection of Isoimmune Serum Into Normal Rabbits.*—The same platelet donor was used for each immunized rabbit during the iso group experiments. After completion of all other studies, the isoimmunized rabbits received a "booster" injection of isoplatelets. One week later, they were again bled. Ten milliliters of serum from each immunized rabbit was mixed with an amount of specific modifying agent, as shown in Table III. The mixture was injected into the donor rabbit. In some instances injection of serum alone was carried out. Platelet count was followed at the same interval of time as described in paragraph 9.

11. *Paper Electrophoretic Studies.*†—A study of electrophoretic migration of serum proteins and protein-bound carbohydrates on paper was conducted in some instances. Electrophoretic migration was obtained by Durrum's technique,[15] with use of barbital buffer, pH 8.6, ionic strength 0.075, and 16 hours' migration. Staining of proteins on the No. 3 Whatman paper was done with Durrum's technique,[15] of protein-bound carbohydrates with Köiw and Grönwall's method.[16]

RESULTS

1. *Heteroimmunization experiments (Table IV).*—Heteroimmunization was carried out with human platelets modified by washing or by incubation with drugs (aminopyrine, quinine, quinidine). The antihuman platelet titers of all recipient rabbits rose to figures higher than 1/1,024 after the second and third immunizing injections, and persisted at such high level for at least two weeks after the last immunization. Titer of *all* immune sera was equally high against unmodified human platelets or human platelets modified by drugs. Also, the absorption of the antibody from the immune sera was always complete whether normal or modified human platelets were used. Thus, when serum from animals immunized with aminopyrine-treated or washed platelets was

*Sharp and Dohme, Philadelphia, Pa.
†This section was contributed by Sergio I. Magalini, M.D., and Elly Moschides, Ph.D., Joseph Stanton Laboratories, Saint Elizabeth Hospital, Boston.

absorbed with washed or aminopyrine-treated platelets, respectively, there was complete absorption of the antibody in both instances. Finally, immune sera showed complete complement fixation when incubated with normal human platelets. In vivo, no drop in platelet count followed the injection of the modifying agents into the corresponding immunized animal. In one case tested, serum electrophoresis showed no change in protein pattern but slight increase of β-globulin-bound carbohydrates.

TABLE IV. RISE IN HETEROPLATELET AND DECREASE IN ISOPLATELET AGGLUTINATING TITER IN THE COURSE OF IMMUNIZATION OF A RABBIT WITH QUININE-TREATED HUMAN PLATELETS (RABBIT 3)

	PLATELET AGGLUTINATING TITER, AGAINST			COMPLEMENT FIXATION TEST	
ANTIGEN	WASHED HUMAN PLATELETS	QUININE-TREATED HUMAN PLATELETS	RABBIT PLATELETS	WITH WASHED HUMAN PLATELETS	WITH WASHED RABBIT PLATELETS
Before immunization	1/32	1/32	1/64 to 1/128*	Weakly positive†	Negative
After immunization	>1/1,024	>1/1,024	1/8*	Strongly positive	Negative

*See text.
†Test performed on serum collected 1 week after first immunizing injection.

An interesting finding occurred in one animal, whose serum exhibited an unexplained high titer agglutinin against all rabbit platelets tested before immunization. The agglutinin titer, however, fell appreciably in the course of immunization with human platelets (Table IV). This finding will be commented on in the discussion of our experimental results.

It appeared, then, that the stimulation by the heterologus (species) antigen greatly overshadowed in this experimental group the possible immunologic stimulation by modified heterologous antigens. Since none of the animals developed an agglutinin against rabbit platelets and the injection of the modifying agent failed to induce thrombocytopenia in vivo, heteroimmunization did not induce iso- or autoimmunization.

2. *Isoimmunization Experiments (Table V and Fig. 1).*—Platelets modified by washing or incubation with drugs, various bacterial filtrates, and Newcastle

TABLE V. BEHAVIOR OF THE PLATELET COUNT IN RABBITS IMMUNIZED WITH ISOPLATELETS MODIFIED BY VARIOUS AGENTS, FOLLOWING THE ADMINISTRATION OF: (A) SPECIFIC MODIFYING AGENT; (B) NONSPECIFIC MODIFYING AGENT. A REPRESENTATIVE SERIES OF EXPERIMENTS

RABBIT NO.	SPECIFIC MODIFYING AGENT INJECTED	DROP IN PLATELET COUNT (%)	NONSPECIFIC MODIFYING AGENT INJECTED	DROP IN PLATELET (%)
22	Aminopyrine	30	--	--
23	Quinine	<25	--	--
24	Quinidine	29	--	--
28	*Streptococcus hemolyticus* filtrate	<25	*Staphylococcus aureus* filtrate	<25
29	*Staphylococcus aureus* filtrate	54	Streptococcus filtrate	<25
30	*Staphylococcus albus* filtrate	50	Quinidine	<25
27	Newcastle disease virus	<25	Allantoic fluid	<25

disease virus were used. Each rabbit was immunized with platelets obtained from one donor rabbit throughout each experiment. Also, the agglutinating titer of the immune animal was usually tested against its donor's platelets.

Uniformly, no agglutination resulted when the immune sera were incubated with normal as well as modified platelets in the presence or not of the modifying agent. Complement fixation tests were also uniformly negative. Platelet counts were taken weekly in some of the rabbits but no significant changes were found. Platelet counts were also followed in samples of blood collected from the immunized animals as well as from normal control rabbits using heparin or Sequestrene-Na_2 as anticoagulant ("Materials and Methods," paragraphs 4 and 7) and incubated with or withous presence of the modifying agent. Figures obtained in these experiments were very erratic. Attempts to detect coating of platelets in the immunized animals by means of their reaction with a guinea pig antirabbit-globulin serum failed because of considerable technical difficulties.

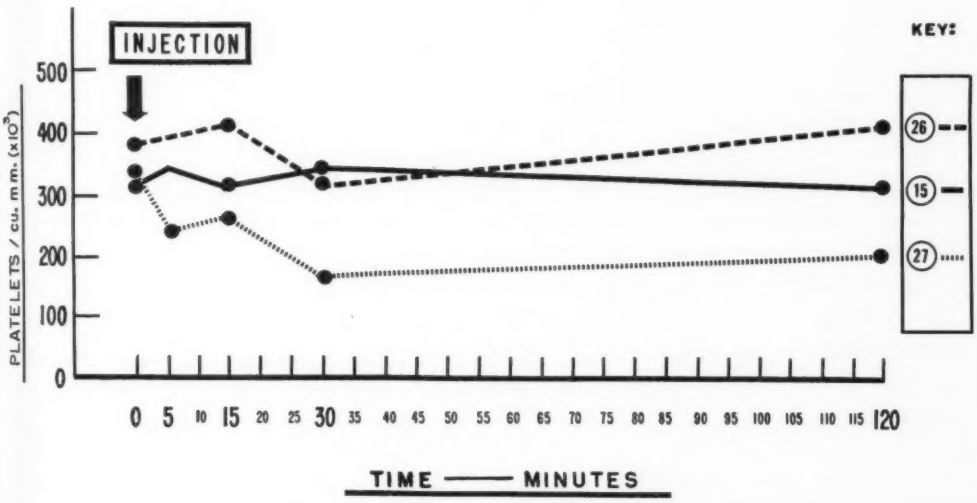


Fig. 1.—Autoimmunization induced in rabbits through isoimmunization. Animals studied were: No. 15, normal rabbit; No. 26, immunized with isoplatelets modified with *Streptococcus hemolyticus* filtrate; No. 27, rabbit immunized with isoplatelets modified with *Staphylococcus aureus* filtrate. All animals received intravenously *Staphylococcus aureus* filtrate. Thrombocytopenia developed only in rabbit No. 27 immunized with specific filtrate-treated platelets.

Nevertheless, the injection of the specific modifying agent in some of the immunized rabbits was followed by significant drop in platelet count. The effect was marked in rabbits which had been immunized with platelets modified with *Staphylococcus albus* and *aureus* filtrates, aminopyrine, and quinidine. Injection of filtrate of *Staphylococcus albus* and *aureus*, aminopyrine, and quinidine in the specifically immunized animals was followed by transitory but significant thrombocytopenia. The injection of a nonspecific agent had no effect (Table V). No thrombocytopenia was obtained in rabbits which had been immunized with quinine, Streptococcus filtrate, and Newcastle disease virus-

treated platelets even when the specific modifying agents were used (Table V). None of the modifying agents caused significant thrombocytopenia when injected alone, even repeatedly, into normal rabbits.

Injection of sera from the immunized rabbits together with the modifying agent into their donors verified the previous results in showing isoimmunization. Thus, serum from rabbits which had received Streptococcus filtrate-treated platelets failed to induce thrombocytopenia when injected in the donor rabbit together with Streptococcus filtrate. It will be remembered that no immunization could be demonstrated in animals treated with platelets modified by Streptococcus filtrate. On the other hand, donors receiving serum from animals immunized with *Staphylococcus albus* and *aureus* filtrate-treated platelets together with the modifying agent developed significant thromocytopenia. The injection of serum or modifying agent alone was without effect.

Thus, we failed to demonstrate by in vitro techniques the development of isoantibodies in rabbits immunized with isoplatelets, possibly because of technical difficulties. In vivo, however, experiments showed that, in many instances, the immunized animals had developed isoantibodies as well as autoantibodies, active only in the presence of the specific modifying agent (Fig. 1). It then appeared that not only had isoimmunization occurred, but that autoimmunization had developed also. *Staphylococcus albus* and *aureus* filtrates, aminopyrine, and quinidine appeared to be particularly effective as modifying agents in the development of iso- and autoimmunization. Development of immunization in this group of animals was also suggested by electrophoretic studies. Thus, paper electrophoresis of serum from rabbits immunized with isoplatelets modified by quinidine showed marked increase in the protein γ globulin fraction, a similar finding which has been reported in other instances of immunization.[17]

Natural isoagglutinins against the donor's platelets before immunization were found in two rabbits only. When 10 ml. of serum from the two experimental animals containing a rabbit platelet agglutinin was injected into a donor animal, thrombocytopenia developed (40 per cent drop in platelet count which lasted longer than two hours). No such phenomenon was observed with other rabbit sera. The finding testified, perhaps, as to true antibody nature of the agglutinin. As immunization progressed, however, the titer against *normal* platelets remained unchanged in one animal and fell in another from one sixty-fourth to one-half. This interesting finding will be commented on in the discussion of this article.

3. *Autoimmunization Experiments (Table VI and Figs. 2 to 5).*—Platelets used in this series were either washed or treated with drugs or bacterial filtrates or Newcastle disease virus. The animals' platelet count taken at regular intervals during immunization failed to show significant variation. In 50 per cent of the animals in various experimental groups, however, platelet-agglutinins of significant titer developed against the rabbits' own platelets or those from normal rabbits. Agglutinins were active in the presence or absence of the specific modifying agent. They were quite short-lived, as they were no longer detectable three months after the last immunizing injection

(Table VI). The development of platelet autoagglutinins in animals failing to develop thrombocytopenia could be considered a paradoxical result. It will be commented upon in the course of the discussion. No agglutinins developed when control rabbits received repeated injections of the modifying agent alone. Complement fixation occurred in only 40 per cent of the animals which had developed platelet agglutinins. This discrepancy may have several explanations. It may be that the concentration of the antibody, although able to cause agglutination, was not sufficient to bind complement, as described for certain bacterial systems.[18] We are also aware that the method used is not very sensitive. In addition, complement was titrated against a different platelet-suspension from the one used in the actual test. This was unavoidable because enough platelets could not be obtained for both purposes from a single rabbit.

TABLE VI. DEVELOPMENT OF AUTO- AND ISOPLATELET ANTIBODIES IN RABBITS RECEIVING AUTOPLATELETS MODIFIED BY VARIOUS AGENTS (REPRESENTATIVE EXPERIMENT)

		PLATELET AGGLUTININ TITER, AGAINST					
		RABBIT'S OWN PLATELETS			NORMAL RABBIT PLATELETS		
RABBIT NO.	MODIFYING AGENT USED	BEFORE IMMUNIZATION	1 WEEK AFTER LAST IMMUNIZING INJECTION	3 MONTHS LATER	BEFORE IMMUNIZATION	1 WEEK AFTER LAST IMMUNIZING INJECTION	3 MONTHS LATER
41	Washing	0	1/8	–	0	1/8	–
42	Aminopyrine	0	1/8	0	0	1/8	0
43	Quinine	0	0	–	0	0	–
44	Quinidine	0	0	–	0	0	–
46	*Streptococcus hemolyticus* filtrate	0	1/16	0	0	1/16	0
47	*Staphylococcus aureus* filtrate	0	0	–	0	0	–
48	*Staphylococcus albus* filtrate	0	0	–	0	0	–
52	Broth	0	0	–	0	0	–
50	Newcastle disease virus	0	1/16	–	0	1/16	–
49	Allantoic fluid	0	1/16	–	0	1/16	–

0 = Negative.
– = Not done.

Injection of the specific modifying agent alone into immunized rabbits caused at times a considerable thrombocytopenia, especially when aminopyrine, quinidine, or bacterial filtrates were used (Fig. 2). The comparison of the thrombocytopenic effect in immunized animals of specific versus nonspecific modifying agents was also of considerable interest. Fig. 3 shows the thrombocytopenic effect of the injection into immunized rabbits of some of the specific and nonspecific agents which had been used to modify the platelets. The duration as well as the intensity of the thrombocytopenic effect seemed to indicate the specificity of the reaction. The effect of some specific modifying agents is shown in Fig. 3, *A*. The injection of the substance was followed by evident prolonged thrombocytopenia. One day after injection of the specific modifying agent, each immune rabbit received a filtrate or a drug which had not been used to modify its own platelets (Fig. 3, *B*). Comparison of findings in Fig.

Fig. 2.—Thrombocytopenia following the injection into immunized rabbits of specific modifying agents (bacterial filtrates). Rabbit 52, immunized with autoplatelets treated with broth; received injection of broth. Rabbit 46, immunized with autoplatelets treated with *Streptococcus hemolyticus* filtrate; received injection of *Streptococcus hemolyticus* filtrate. Rabbit 47, immunized with autoplatelets treated with *Staphylococcus aureus* filtrate; received injection of *Staphylococcus aureus* filtrate. Rabbit 48, immunized with autoplatelets treated with *Staphylococcus albus* filtrate; received injection of *Staphylococcus albus* filtrate. Rabbits 46, 47, 48 developed persistent thrombocytopenia.

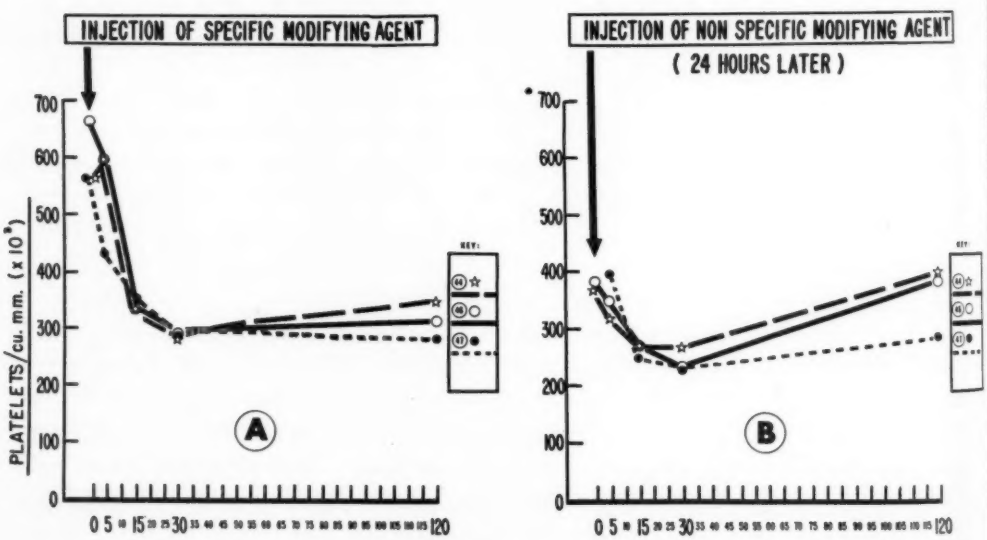


Fig. 3.—Thrombocytopenic effect of specific and nonspecific platelet modifying agents in autoimmunized rabbits. Rabbit 44, immunized with autoplatelets modified by quinidine, received quinidine in *A* and *Streptococcus hemolyticus* filtrate in *B*. Rabbit 46, immunized with autoplatelets modified by *Streptococcus hemolyticus* filtrate received *Streptococcus hemolyticus* filtrate in *A* and *Staphylococcus aureus* filtrate in *B*. Rabbit 47, immunized with autoplatelets modified by *Staphylococcus aureus* filtrate, received *Staphylococcus aureus* filtrate in *A* and *Staphylococcus albus* filtrate in *B*. It may be noted that Rabbit 47 in *B* showed a more prolonged response than either No. 44 or No. 46. This rabbit received filtrates from *Staphylococcus aureus* (to which it was immunized) and *albus* (see text). Injection of specific modifying agents was followed by sustained thrombocytopenia.

3, *A* and *B* shows that the thrombocytopenic effect of the specific agent was still evident 24 hours later. Also, thrombocytopenic effect obtained with nonspecific modifying agents was less pronounced and, especially, of much shorter duration. Of some significance is the fact that Rabbit 47, immunized with autoplatelets treated with *Staphylococcus aureus*, reacted to the injection of the filtrate from *Staphylococcus albus* with a significant thrombocytopenia. This may suggest a cross immunization due to closely related modifying agents. The question of whether some of the findings in Fig. 3, *B* might have been due to the short interval between injection of the specific and the nonspecific modifying agent was answered negatively by another experiment. Quinidine was injected into an animal which had been immunized with its own quinine-treated platelets, and which had not received any kind of injection for one week. The animal's platelet count was at the same level as found before immunization. The effect obtained was similar in magnitude to that shown in Fig. 3, *B* for other nonspecific modifying agents (Fig. 4). The duration of immunization appeared to be short-lived. When rabbits immunized with their own quinidine-treated platelets received an injection of the drug 3 months after the last immunization, thrombocytopenia could no longer be obtained.

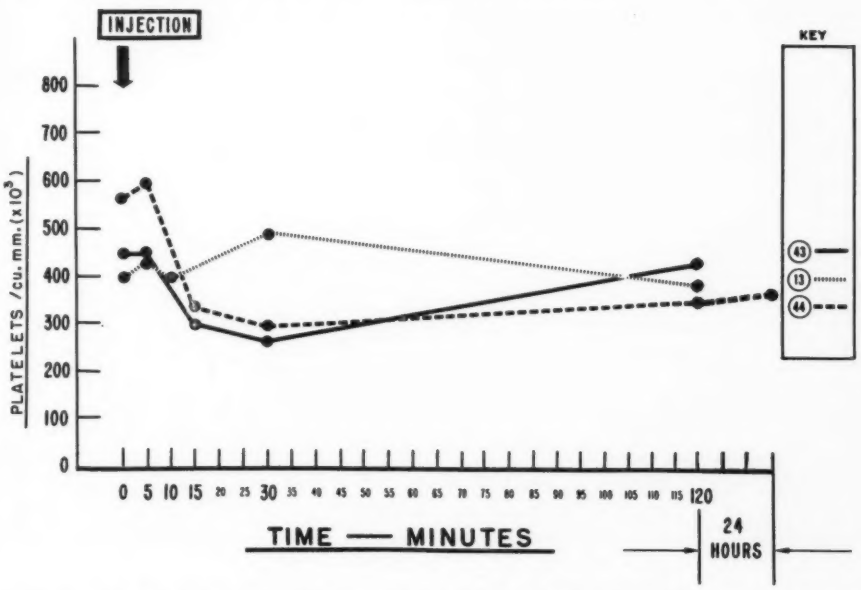


Fig. 4.—Specific and aspecific thrombocytopenia effects of platelet modifying agent injected in immunized rabbits. Quinidine was administered to: Rabbit 44, immunized with own quinidine-treated platelets, Rabbit 43, immunized with own quinine-treated platelets, Rabbit 13, a normal control. The thrombocytopenic response was more prolonged in the specifically immunized animals. In the rabbit immunized with quinine-treated platelets, quinidine also caused thrombocytopenia, with return, however, of platelet count to normal within two hours.

No indication of immunization was observed in animals whose platelets had been treated with Newcastle disease virus. This finding was somewhat paradoxical, since a drop in platelet count resulted when sterile allantoic fluid was injected as the specific modifying agent in one rabbit, which had received its

own platelets treated with this agent. One wonders whether phenomena of absorption between virus and constituents of the allantoic fluids might have impaired the antigenic property of the latter. On the other hand, this finding also emphasizes the question of the individual response to immunization. Thus, auto- and isoagglutinins developed in one rabbit receiving platelets modified by washing with saline and standing, but not in rabbits which received quinine, quinidine, and *Staphylococcus albus* and *aureus* filtrates. Washing of platelets and incubation were part of the procedure for modification of platelets with these agents.

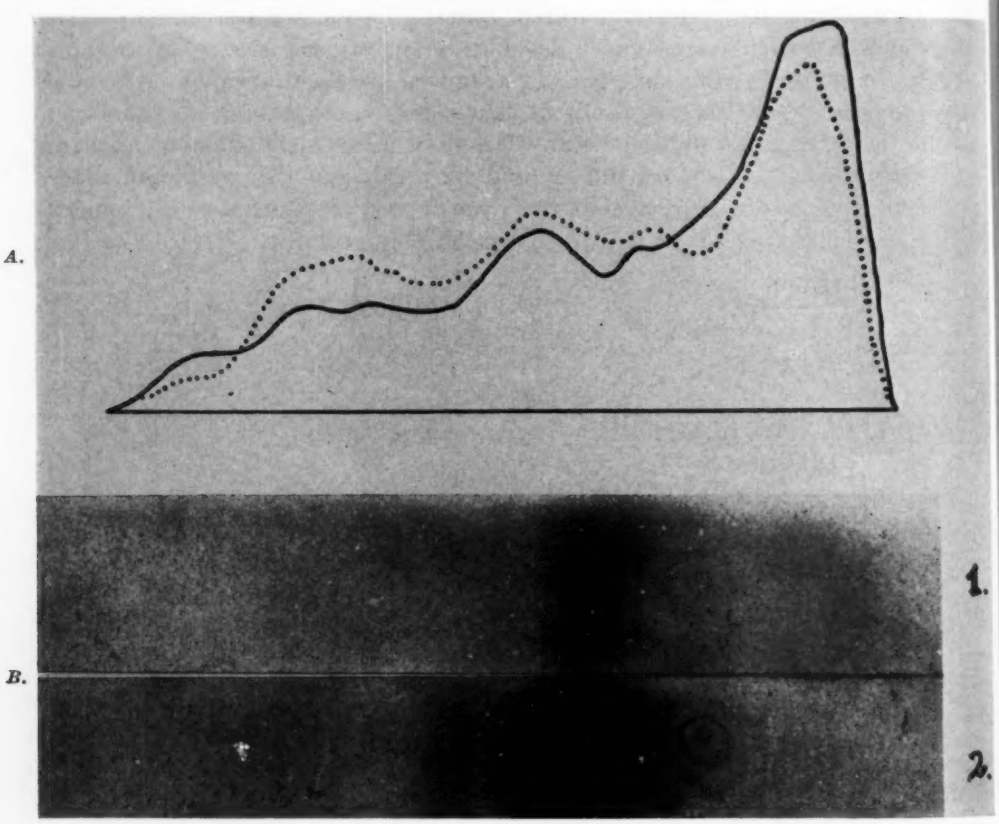


Fig. 5.—Electrophoretic changes in rabbit serum, after immunization with the animal's own platelets modified by *Streptococcus hemolyticus* filtrate (Rabbit 46). Volume of serum was delivered from standard capillary pipette from standard height. *A*, Electrophoretic distribution of serum proteins (optical density readings) before (full time) and after (dotted line) immunization: note increase in γ fraction after immunization. *B*, Protein-bound carbohydrates before (*1*) and after (*2*) immunization: note increase in α_2 β, and γ-globulin-bound carbohydrates.

Again electrophoretic studies showed changes similar to these found in animals during other processes of immunization (Fig. 5, *A* and *B*). They consisted in increase in γ and α_2 fraction of proteins and carbohydrates bound to the same globulin fractions. Rabbits with low titers of agglutinin did not show electrophoretic changes.

A number of conclusions were reached with these experiments: (1) treatment of the animal's own platelets with drug or bacterial filtrates often made them antigenic to their own organism. Autoantibodies developed which were active in vitro in the presence or in the absence of the platelet modifying agent; (2) moreover, injection into the immune animal of the specific agent used for modification of platelets caused significant thrombocytopenia, while much less pronounced and more fleeting effect followed administration of a nonspecific agent; (3) in the course of autoimmunization, isoimmunization developed. This conclusion was justified by the finding that iso antibodies became demonstrable in the autoimmunized animals and active in vitro against normal rabbit platelets in the presence or absence of the modifying agent.

DISCUSSION

The variability and impredicability of results during immunization experiments are well known. It is then understandable that our findings were not constant. Results quoted here are those of successful experiments. This may appear an obstacle in deriving conclusions unless one recognizes the limitations of our experimental approach and the fact that, as in humans, individual susceptibility is indispensable for the development of immunologic states. Thus, there is no doubt that quinidine is responsible for certain instances of acute thrombocytopenic purpura in humans. Yet, quinidine-induced thrombocytopenic purpura is so infrequent that many a cardiologist with large experience in the use of the drug has not seen a single case. This consideration influenced heavily the planning of our experiments. Two alternatives appeared possible. One, to select a single platelet modifying agent and see how often its use would induce an immune response. The other, to try several platelet modifying agents. The second approach was selected because we felt it would be more likely to supply significant results without bringing the number of experiments to impractical figures.

A basic problem became obvious in the course of these experiments: did the results obtained indicate a specific immunologic reaction or, rather, a nonspecific phenomenon? Some of our findings might have been explained on the basis of a nonspecific reaction. As an example, the occurrence of thrombocytopenia following injection of the specific immunizing agent in the exposed animals may not have been, in itself, a proof of the existence of a specific immune state. Thrombocytopenia of a very transitory nature is known to be one of the nonspecific responses of immunized individuals upon exposure to the specific antigen; thus, patients suffering from food or pollen allergy may develop transitory thrombocytopenia when exposed to the specific allergen, probably as expression of a mild anaphylactic state induced by the introduction of a foreign material. The thrombocytopenia that we observed in our immune animals, however, was prolonged and quite different from the transitory platelet fall observed in animals receiving an antigen to which they had not been previously exposed.

A number of observations which are not given in detail for considerations of space also emphasized the specificity of our findings. It is quite likely that the development of a specific antibody against one formed element of the blood may, by a mechanism of cross-immunization, be accompanied by the appearance of antibodies against other blood and tissue cells. For this reason, attention was given to behavior of hematocrit and white cell count in immune animals receiving the specific antigen. No reduction in hematocrit nor significant leukopenia was observed in these experiments. Thus, although the finding of anemia or leukopenia might not have invalidated the significance of our results, the specificity of the antibody obtained was then apparent. Perhaps this was due to the low degree of antigenic stimulation, also suggested by the low titers of platelet antibodies obtained in most experiments. Also, repeated administration of the modifying agent alone, which probably coupled with plasma proteins, did not induce thrombocytopenia on further exposure. In unpublished work of a few years ago[19] red cells modified by drugs and bacterial filtrates were injected repeatedly into rabbits. The administration of the modifying agent shortly after completion of the immunization failed to induce appreciable anemia or significant thrombocytopenia. These negative findings seem to support our contention that the development of platelet antibodies in some of our experimental animals was truly a specific immunologic reaction. The positive findings in favor of this conclusion are obvious from the review of our results.

The experiments presented in this article are based on the concept that platelets are antigenic and that their antigenicity may be modified by the action of exogenous agents. There is ample evidence to indicate that platelets are antigenic. Thus, heteroantigenicity of platelets has long been demonstrated[20]; the hypothesis that platelets are isoantigenic has received considerable support from the recently discovered evidence for individual platelet groups.[5, 21, 22] It is also probable that platelets are autoantigenic, since specific autoantibodies may be found in patients with idiopathic thrombocytopenic purpura. The production by an individual of antibodies against his own platelets seems to imply that platelets have been modified antigenically. The very chemical complexity of platelets emphasizes the opportunity for their antigenic modification. It is known that antigenic specificity is closely related to chemical structure. A change in structure, then, may influence the antigenic properties and even induce a new antigenic specificity.[23, 24] In our experiments, animals receiving their own platelets after proper treatment developed auto- as well as isoantibodies (agglutinins). Since modified platelets, likely different from intact platelets, represented the antigenic stimulus, autoimmunization and isoimmunization must have developed during the process of antibody formation against modified platelets. This finding is sufficient evidence that the antigenic nature of platelets was modified through exogenous influences, perhaps affecting the fine platelet structure.

There is little doubt that specific autoimmunization was at times achieved, especially in the autoimmunization experiments. Auto- and isoplatelet agglutinins developed in this group. Fixation of complement was positive in many

instances. Also, electrophoretic changes appeared in the serum of the immunized animals not unlike those seen in other types of experimental immunization[17] and in cases of immune idiopathic thrombocytopenic purpura in humans.[25] Injection of agents used to modify platelets into the specifically immunized rabbits induced a drop in platelet count. Striking indeed was the extreme specificity of the thrombocytopenic effect which followed the injection of modifying agents in the immunized animals. When the specific modifying agent was injected, thrombocytopenia was considerable and prolonged; when an aspecific one was use, thrombocytopenia was limited and of very short duration. A certain "group specificity," however, seemed to exist when animals immunized with their own platelets modified by *Staphylococcus aureus* filtrate developed significant thrombocytopenia upon injection of *Staphylococcus albus* filtrate.

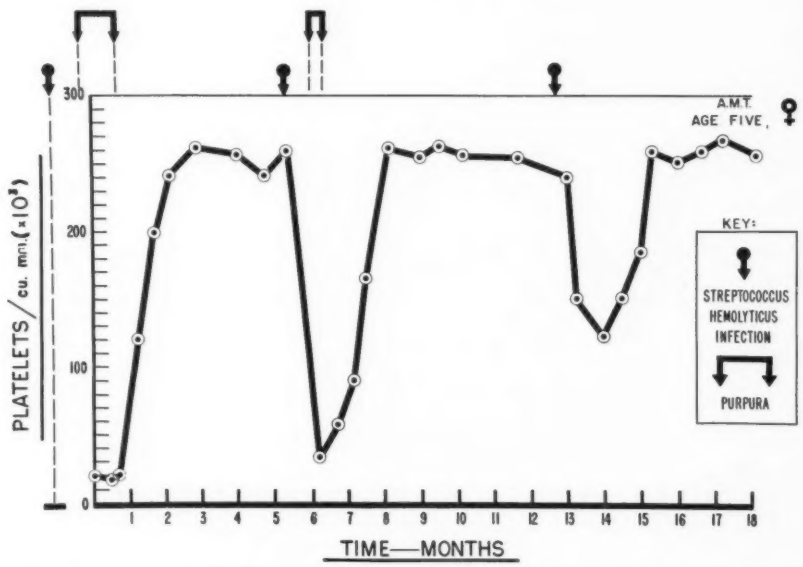


Fig. 6.—Recurrent acute "idiopathic" thrombocytopenic purpura in a child, probably due to bacterial activity. The child, A.M.T., aged 5 years, female, developed 2 episodes of purpura following sore throat. *Streptococcus hemolyticus* was isolated in each instance by throat culture. A third episode of sore throat was not accompanied by clinical purpura, but the platelet count dropped temporarily. Again throat culture isolated *Streptococcus hemolyticus*. Aspirin was given during all acute episodes. Patient also received Chloromycetin during the first episode; Gantrisin later.

Results in the auto- and isoimmunized group of animals were of considerable interest with reference to the pathogenesis of immune human thrombocytopenia. Results in the auto group have already been summarized. An additional comment appears, however, necessary. Curiously enough, platelet autoagglutinins developed in animals which failed to show thrombocytopenia. This result is perhaps comparable to findings in acute and chronic immune thrombocytopenic purpura in humans. In acute human thrombocytopenia, platelet agglutinins may rarely be found; but, when present, may persist long after the platelet count has returned to normal. In chronic human thrombo-

cytopenic purpura, platelet agglutinins are also found during phases of remission, when the platelet count is closer to normal values. Following splenectomy, thrombocytopenia may remit when a platelet agglutinin may still be detected in the patient's serum.[4] Also, their serum may still induce thrombocytopenia when injected into normal individuals.[5] These considerations, together with the fact that the animals' platelet agglutinin titer was low may explain the coexistence of a normal platelet count and a platelet agglutinin in the same subject.

Results in the iso group are briefly reviewed here. No antibodies demonstrable in vitro developed. Injection of the specific agent used to modify platelets into the immunized animal, however, was followed by thrombocytopenia in many instances. Findings in this group could perhaps be correlated with observations in the self-limited acute variety of idiopathic thrombocytopenic purpura, a disease which is often preceded by bacterial or viral infections or by administration of drugs. Detection of auto- or isoplatelet agglutinins is here extremely rare, but a careful follow-up shows that administration of drugs or contact with a specific bacterial agent *may* cause relapse of thrombocytopenia (Fig. 6). Also, the simultaneous injection of serum of isoimmunized animals and specific modifying agent into normal animals induced thrombocytopenia. This last result finds almost exact replica in studies of quinine-induced purpura in humans.[25]

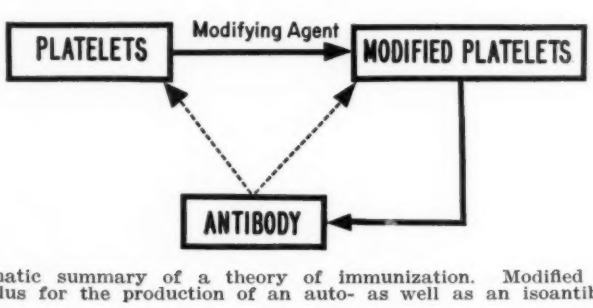


Fig. 7.—A schematic summary of a theory of immunization. Modified platelets are the antigenic stimulus for the production of an auto- as well as an isoantibody (see text).

In both auto- and isoimmunized animals, auto- and isoantibodies developed simultaneously. It may be concluded that isoimmunization and autoimmunization occurred together.* It is interesting to note that simultaneous occurrence of auto- as well as of isoantibodies (agglutinins) is found in two pathologic conditions in humans. The first is the idiopathic variety of chronic thrombocytopenic purpura where the pathogenetic mechanism is likely to be one of autoimmunization, at least in some instances. The second is the appearance of iso-

*The cross-development of auto- and isoantibodies in the course of immunization is of considerable interest. Current theories about the production of antibodies emphasize the influence of the antigen on the formation of the antibody-globulin molecule and the resulting complementariness in structure.[27, 28] However, antibodies to a given antigen with one determinant group are not always identical but vary around a main pattern.[24, pp. 270-271] Antigens with strongly acting groups are postulated to give rise to less specific antibodies.[28] Modified platelets may represent such an antigen. Thus, in autoimmunization, once platelets are used which are antigenic, antibodies might then develop which may attack normal iso- as well as autoplatelets (Fig. 7). In the case of isoimmunization a similar mechanism may be at play. In each instance, the antigenic structure of the modified platelets probably differs from that of the normal platelets only in some groups or parts of the antigenically active surface. An antibody is then likely to form, which reacts also with some of the antigen structures common to normal platelets. This highly hypothetical theory is only offered as a matter of speculation.

and, later, of autoplatelet agglutinins in patients with the megakaryocytic type of thrombocytopenia who have received multiple platelet transfusions. This is likely to be the end result of isoimmunization on the basis of the administration of incompatible platelets. In both instances, the immunologic nature of the antibody production mechanism is obvious and lends support to the hypothesis that in our animals also production of platelet agglutinins was due to specific antigenic stimulation.

A finding of interest was noted in the hetero- and isoimmunized groups of animals. A few animals exhibited naturally occurring isoantibodies, which fell in titer or disappeared altogether in the course of specific hetero- and isoimmunization. The finding has no simple explanation. At least in the isoimmunized group, the absorption of the natural antibody by the modified rabbit platelets injected might have contributed to the drop of its titer. Also, perhaps, during the formation of a strong antibody the production of a weaker, naturally occurring antibody was greatly diminished. With reference to this hypothesis it is known that, even under extreme conditions, there is a certain limit to the ability of the organism to produce plasma proteins[29] and that during immunization with different antigens or antigens with different properties, a stronger antigen may dominate antibody globulin formation.[30, 31]

SUMMARY

1. A attempt was made to induce platelet antibody production in rabbits by injection of antigenically modified platelets. Modification of platelets was attempted by: (a) washing and standing; (b) incubation with drugs (aminopyrine, quinine, quinidine); (c) treatment with bacterial filtrates (*Streptococcus hemolyticus* and *Staphylococcus albus* and *aureus*); (d) treatment with Newcastle disease virus. Treated platelets were injected into 3 groups of rabbits; one received human platelets (heterologous immunization), one received platelets from normal rabbits (isoimmunization), and the third group received their own platelets (autoimmunization). Development and specificity of platelet agglutinins were evaluated by standard immunologic methods in vivo and in vitro.

2. High titer heteroimmune agglutinins developed constantly against human platelets. There was not, however, evidence for a specific antigenicity of modified versus nonmodified platelets. In one animal a naturally occurring isoantibody decreased in titer as the heteroantibody developed.

3. Autoantibodies and isoantibodies were often detected in the serum of rabbits receiving their own modified platelets in the presence or in the absence of the modifying agent. Animals receiving isoimmunization failed, however, to develop specific antibodies in vitro. Changes in protein and glycoprotein electrophoretic pattern were noted in the serum of auto- and isoimmunized rabbits, which were similar to those obtained in other instances of experimental immunization. When the specific modifying agent was injected into auto- or isoimmunized rabbits, significant thrombocytopenia followed in many instances. The

state of immunization was short-lived (less than 3 months). Results of our experiments indicated the simultaneous development of isoimmunization and autoimmunization in the course of the injection of modified auto- or isoplatelets.

4. Experimental results were not constant and reproducible in all animals of any given group. This is quite in agreement with the observations in humans, where only a few individuals, among many, develop thrombocytopenia after exposure to drugs, bacteria, and viruses.

5. Some of the findings obtained in the course of these studies could be applied to the interpretation of the pathogenesis of some thrombocytopenic states in man.

REFERENCES

1. Stefanini, M.: Immuno-hematologic Disorders. A Theory of Disease, Proc. Inst. Med. Chicago **20:** 335-346, 1955.
2. Kaznelson, P.: Thrombolytische Purpura, Ztschr. klin. Med. **87:** 133-164, 1919.
3. Doan, C. A.: Hypersplenism, Bull. New York Acad. Med. **25:** 625-650, 1949.
4. Stefanini, M., Dameshek, W., Chatterjea, J. B., Adelson, E., and Mednicoff, I. B.: Studies on Platelets. IX. Observations on the Properties and Mechanism of Action of a Potent Platelet Agglutinin Detected in the Serum of a Patient With Idiopathic Thrombocytopenic Purpura (With a Note on the Pathogenesis of the Disease), Blood **8:** 26-64, 1953.
5. Harrington, W. J., Sprague, C. C., Minnich, V., Moore, C. V., Ahlvin, R. C., and Dubach, R.: Immunologic Mechanisms in Idiopathic and Neonatal Thrombocytopenic Purpura, Ann. Int. Med. **38:** 433-469, 1953.
6. Stefanini, M.: Studies on Platelets. XIII. Idiopathic Thrombocytopenic Purpura. Immunologic Aspects, Sang **26:** 83-112, 1955.
7. Frank, E.: Die essentielle Thrombopenie. I. Klinisches Bild. II. Pathogenese, Berl. klin. Wchnschr. **52:** 454-458 and 490-494, 1915.
8. Dameshek, W., and Miller, E. B.: The Megakaryocytes in Idiopathic Thrombocytopenic Purpura, a Form of Hypersplenism, Blood **1:** 27-51, 1946.
9. Harrington, W. J., Minnich, V., Hollingsworth, J. W., and Moore, C. V.: Demonstration of a Thrombocytopenic Factor in the Blood of Patients With Thrombocytopenic Purpura, J. Lab. & Clin. Med. **38:** 1-10, 1951.
10. Gardner, E., Jr., Wallace, J. H., Dodd, M. C., and Wright, C-S.: Antigenically Modified Red Cells in Chickens Infected With Newcastle Disease, Proc. Soc. Exper. Biol. & Med. **87:** 253-257, 1954.
11. Stefanini, M.: The Diagnosis of Hemorrhagic Diseases, Bull. New England Med. Center **12:** 102-115, 1950.
12. Stefanini, M., and Dameshek, W.: The Hemorrhagic Disorders, New York, 1955, Grune & Stratton, page 286.
13. Adelson, E., and Stefanini, M.: Studies on Platelets. VI. Demonstration and Characterization of a Heterologous (Forssman) Platelet Agglutinin, Blood **7:** 700-709, 1952.
14. Todd, J. C., and Sanford, A. H.: Clinical Diagnosis by Laboratory Methods, Philadelphia, 1948, W. B. Saunders Co., page 802.
15. Block, R. T., Durrum, E. L., and Zweig, A.: Paper Chromatography and Paper Electrophoresis, New York, 1955, Academic Press, Inc., page 407.
16. Köiw, E., and Grönwall, A.: Staining of Protein-bound Carbohydrates After Electrophoresis of Serum on Filter Paper, Scandinav. J. Lab. & Clin. Invest. **4:** 244-246, 1952.
17. Weimer, H. E., Jameson, E., Redlich-Moshin, J., Quinn, F. A., and Nelson, E. L.: Serum Glycoprotein Concentration Following Immunization in the Rabbit, Proc. Soc. Exper. Biol. & Med. **88:** 571-574, 1955.
18. Kabat, E. A., and Mayer, M. M.: Experimental Immuno-Chemistry, Springfield, Ill., 1948, Charles C Thomas, Pub., page 131.
19. Stefanini, M.: Unpublished results. Dept. Biochemistry, Marquette University, 1948–1949.
20. Marino, F.: Recherches sur les plaquettes du sang, Compt. rend. Soc. Biol. **58:** 194-196, 1905.
21. Stefanini, M., Plitman, G. I., Dameshek, W., Chatterjea, J. B., and Mednicoff, I. B.: Studies on Platelets. XI. Antigenicity of Platelets and Evidence for Platelet Groups and Types in Man, J. Lab. & Clin. Med. **42:** 723-738, 1953.
22. Gurevitch, J., and Nelken, D.: ABO Groups in Platelets, J. Lab. & Clin. Med. **44:** 562-570, 1954.

23. Obermeyer, F., and Pick, E. P.: Ueber die chemischen Grundlagen der Arteigenschaften der Eiweisskörper, Wien. klin. Wchnschr. **19:** 327-334, 1906.
24. Landsteiner, K.: The Specificity of Serological Reactions, Cambridge, 1945, Harvard Univ. Press, p. 157.
25. Magalini, S. I., Stefanini, M., de Magalhaes, N. M., Angelopoulos, B., and Kistner, S. A.: Electrophoretic Distribution of Serum Glycoproteins in Some Hemorrhagic States, J. LAB. & CLIN. MED. **47:** 923-937, 1956.
26. Steinkamp, R., Moore, C. V., and Doubek, W. G.: Thrombocytopenic Purpura Caused by Hypersensitivity to Quinine, J. LAB. & CLIN. MED. **45:** 18-29, 1955.
27. Breinl, F., and Haurowitz, F.: Chemische Untersuchung des Präzipitates aus Hämaglobin und Antihämoglobin-serum und Bemerkungen über die Natur der Antikörper, Z. Physiol. Chemie. **192:** 45-57, 1930.
28. Pauling, L.: A Theory of the Structure and Process of Formation of Antibodies, J. Am. Chem. Soc. **62:** 2643-2657, 1940.
29. Rotscheid-Robbins, F. S., Miller, L. L., and Whipple, G. H.: Maximal Hemoglobin and Plasma Protein Production Under the Stimulus of Depletion, J. Exper. Med. **82:** 311-313, 1945.
30. Doerr, R., and Berger, W.: Globulin und Albumin aus dem selben Blut Serum als immunisatorische Antagonisten, Biochem. Ztschr. **131:** 13-19, 1922.
31. Irwin, M. R., and Golden, A.: Individual Response in Rabbits to Immunization With a Complex Antigen, J. Infect. Dis. **70:** 119-125, 1942.

CANINE EXPERIMENTAL NEPHROSIS

GUNNAR B. STICKLER, M.D.,* KHALIL G. WAKIM, M.D.,** AND
BERNARD F. MCKENZIE, M.S.***
ROCHESTER, MINN.

AMONG many investigators working on experimental nephropathy, Heymann[1, 2] was able to produce renal disease in rats and puppies, which simulated many aspects of the nephrotic syndrome in man. This was achieved by injecting nephrotoxic serum (NTS) from rabbits which had been sensitized to rat or dog kidney.

The marked changes found in the electrophoretic distribution of serum and urine proteins in the human nephrotic syndrome suggested the study of the changes occurring in the experimental disease. Hieronymi and associates[3] studied by microelectrophoresis the changes of the serum protein fractions in rats after administration of NTS, which was prepared according to the method of Masugi. No quantitative serum protein determinations were reported. They found a decrease in relative albumin concentration within 48 hours. After one week the relative amounts of albumin present in the serum remained low, while the relative levels of globulins increased. After 3 to 4 weeks, a normal distribution of serum proteins was found. Moench and associates[4] made similar observations on four rabbits which were given NTS prepared from ducks sensitized to rabbit kidney. Following the injection of NTS the serum albumin values were reduced, while the alpha-globulin and beta-globulin values increased. The gamma-globulin levels remained the same. The total protein values in the serum and the protein concentrations in the urine were not reported.

Drabkin and Marsh[5] and McCrory[6] reported similar findings in the serum of rats made nephrotic according to the method of Heymann. McCrory found a less marked decrease in the albumin concentrations than Drabkin and Marsh. These authors reported the findings in very small series.

Heymann,[2] reporting on experimental nephrosis in five puppies, found a daily protein excretion between 0.5 and 6.0 Gm. The total serum protein values decreased from 4-5.3 Gm. per 100 ml. to 2.6 Gm. per 100 ml., while

From the Mayo Clinic and Mayo Foundation, Rochester, Minn. The Mayo Foundation is a part of the Graduate School of the University of Minnesota.

This is an abridgment of the thesis submitted by Dr. Stickler to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Pediatrics.

Received for publication May 14, 1956.

*Fellow in Pediatrics, Mayo Foundation.

**Section of Physiology, Mayo Clinic and Mayo Foundation.

***Section of Biochemistry, Mayo Clinic and Mayo Foundation.

the serum cholesterol rose from 105-165 mg. per 100 ml. to 350 mg. per 100 ml. No electrophoretic studies were reported. At the height of the disease ascites developed in two dogs, but no anasarca was observed.

In the present study we used young dogs (5 to 9 months of age) for the production of experimental nephrosis. Quantitative determination of serum and urinary proteins, serum cholesterol, blood urea, and urine output was made throughout the period of study. The blood pressure was also recorded. The variations that were found in the experimental disease of dogs were very similar to those observed in the nephrotic syndrome in man. A close inverse relation between the serum albumin and cholesterol levels was observed.

METHOD

The nephrotoxic serum was prepared according to the directions of Heymann[1, 2] with minor modifications. The kidneys from dogs weighing 10 to 15 kilograms were removed under aseptic conditions, the renal artery was cannulated and the kidney was perfused with sterile saline solution under a hydrostatic pressure of about 1 meter until the organ appeared grossly free of blood. The kidneys were then decapsulated and cut into small slices. A volume of saline solution, equal to weight of the kidney slices was added, and the mixture was homogenized by placing it in a Waring Blendor for 5 minutes. Following this, 3 ml. of toluene and approximately 1,000 units of penicillin per milliliter of kidney suspension were added. The suspension was placed in an incubator at 37° C. for 24 hours. At the end of this period, the material was cultured. Contaminated material was discarded.

For sensitization with this dog kidney suspension, rabbits weighing between 2 and 2.5 kilograms were used. The rabbits were given every second day a single intraperitoneal injection of the material according to the following schedule: first dose 10 ml. of a 10 per cent suspension; second dose 10 ml. of a 15 per cent suspension; nine doses of 10 ml. of a 20 per cent suspension. One week after the last injection the rabbits were exsanguinated under anesthesia by cannulating the femoral artery. The blood was pooled and centrifuged under sterile conditions. The serum obtained was heated for 30 minutes at 56° C. and then stored frozen until needed.

For the injection with NTS, dogs between 5 and 9 months old, weighing between 5 and 10 kilograms, were used. The total protein in serum and urine was measured by the Kingsley[7] procedure. Paper electrophoresis in serum and urine was carried out on the Durrum[8] apparatus, and the paper strips were quantitated by the technique of Grassmann, Hannig, and Knedel.[9] All values for protein and its fractions are presented in grams per 100 ml. of serum. The total serum cholesterol was determined according to the method of Zlatkis and associates[10] and the blood urea by using the method of Van Slyke and Cullen[11] with the modification of Sobel and associates.[12] The systolic and diastolic blood pressures were recorded galvanometrically on photosensitive paper by means of a strain-gauge manometer[13] connected to a needle inserted in the femoral artery. The animals were trained for this procedure.

The animals were individually placed in separate metabolism cages and received a standard diet throughout the course of the experiment; each was given 1 pound per day of Hill's dog food, which contained horse meat, ground green bone, cracked wheat, and cracked pearl barley. The minimal content of protein was 12 per cent and of fat 3 per cent. The food also contained the essential vitamins.

The urine was collected over 24-hour periods, and the total amount of urine, the protein concentration, and the content of the urinary sediment were determined. The animals were weighed every second day. Two milliliters of blood was drawn every 2 to 4 days for the determination of the total serum cholesterol, total serum protein, and its constituent electrophoretic fractions. In a group of eight animals blood urea was determined every 8 days.

After a control period, lasting 5 to 7 days, during which control values for serum and urine were obtained, the intravenous administration of NTS began. In Group 1, consisting of nine animals, each animal received a total of 6.15 ml. per kilogram of NTS. In Group 2 consisting of four animals, each animal received a total of 3.0 ml. per kilogram. In Group 1 the total amount to be used was divided into nine doses and was given over a 3-day period, and in Group 2 the total was divided into five doses and given over 2 days. One hour before each injection of NTS, each animal was given 25 mg. of tripelennamine hydrochloride (Pyribenzamine Hydrochloride, Ciba) intramuscularly. This was given to reduce the primary toxicity of the serum.

RESULTS

Control Period.—In order that control values of the variables to be studied during the experimental disease might be obtained, blood was drawn three to five times over a period of 7 days before the injection of NTS. The means (and their standard errors) of the values found for each animal during the control period were calculated. In turn, the means of these means were calculated. The values obtained during the control period were considered to represent normal values for dogs between 5 and 9 months old. They are presented in Table I.

TABLE I. NORMAL VALUES (15 ANIMALS)

	MEAN OF MEANS	RANGE OF MEANS	RANGE OF STANDARD ERRORS OF MEANS
Serum cholesterol (mg./100 ml.)	203	168-230	2.9-11.4
Total serum protein (Gm./100 ml.)	4.44	3.90-5.95	0.06-0.42
Serum albumin (Gm./100 ml.)	1.96	1.32-2.77	0.02-0.28
Serum alpha$_1$ globulin (Gm./100 ml.)	0.41	0.29-0.51	0-0.04
Serum alpha$_2$ globulin (Gm./100 ml.)	0.64	0.51-0.90	0.02-0.06
Serum beta globulin (Gm./100 ml.)	1.01	0.59-1.94	0.02-0.18
Serum gamma globulin (Gm./100 ml.)	0.47	0.29-0.70	0.005-0.15
Blood urea (mg./100 ml.)	24	17.5-33.0	
Blood pressure, systolic (mm. Hg)	170	146-185	
Blood pressure, diastolic (mm. Hg)	102	76-125	
Weight (Kg.)	7.5	4.9-10.1	0-0.11
Urine volume (ml./24 hr.)	237	178-365	8.9-79.0
Urine protein (Gm./24 hr.)	0.039	0-0.130	0.0007-0.044

FINDINGS DURING THE COURSE OF THE DISEASE

Group 1.—

Clinical findings: Following the course of injections of NTS, seven of the nine animals had anorexia lasting from 2 to 9 days; three of them vomited for 2 to 3 days. In the seven animals no further difficulties were encountered. Following the period of anorexia and vomiting the animals appeared clinically normal and active. One animal died on the eighth day. In one instance ascites developed on the eighteenth day, on the nineteenth day generalized pitting edema was observed, and the animal died on the twenty-fifth day after the injection of NTS.

Laboratory findings: All findings are given as percentage changes from the mean value which was established during the control period. For each day of observation the mean value of the individual observations and its standard error were calculated. The mean curves and the standard errors of the mean

for the variables observed are plotted in Figs. 1, 3, and 4. On the abscissa the dates are given in days beginning on the first day of injection of NTS.

Serum albumin (Fig. 1): Proteinuria was present after the first day of NTS injections and was accompanied by a decrease in serum albumin. The time interval during which the lowest albumin level was reached varied between 4 and 24 days but was usually not longer than 12 days. The maximal average

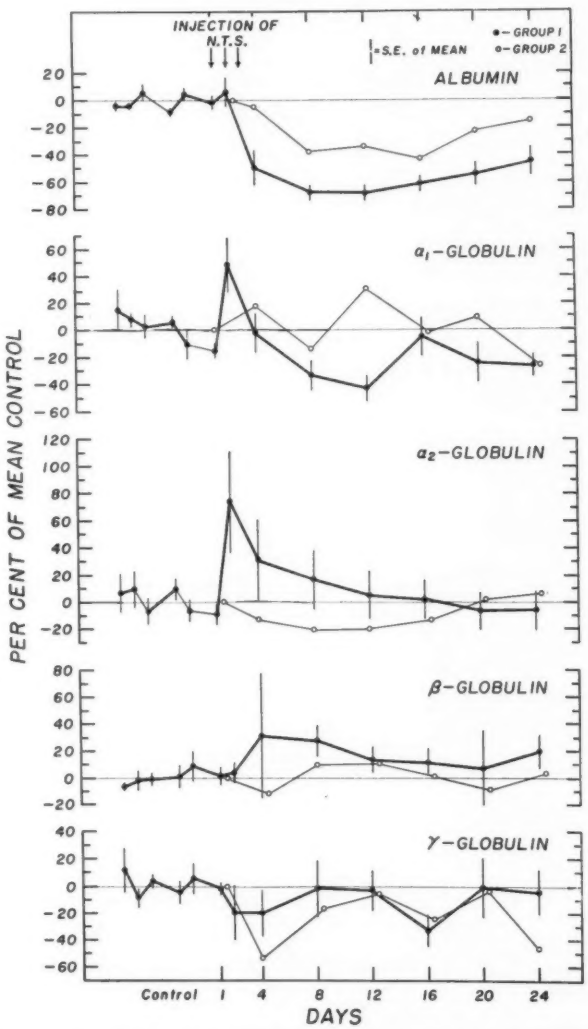


Fig. 1.—The serum protein fractions in experimental nephrosis; changes expressed as per cent of mean control. Group 1 contained nine animals; Group 2 contained four animals.

reduction was about 68 per cent below the control value. The lowest levels found in the serum varied between 0.11 and 0.81 Gm. per 100 ml. After 8 to 12 days the amount of albumin excreted in the urine decreased gradually; normal serum albumin levels were found in five animals after 40 days and in two animals after 80 days.

Serum globulins: During the period of NTS injections the alpha$_1$ globulin rose significantly. Simultaneously with a relatively high excretion of this protein in the urine the amount in the serum fell and remained low throughout the rest of the observation period. The lowest concentration of alpha$_1$ globulin was recorded within 12 days and amounted to a decrease of 42.5 per cent of the mean of the control values. These lowest concentrations ranged between 0.04 and 0.27 Gm. per 100 ml. The range of the control values was 0.29 to 0.51 Gm. per 100 ml.

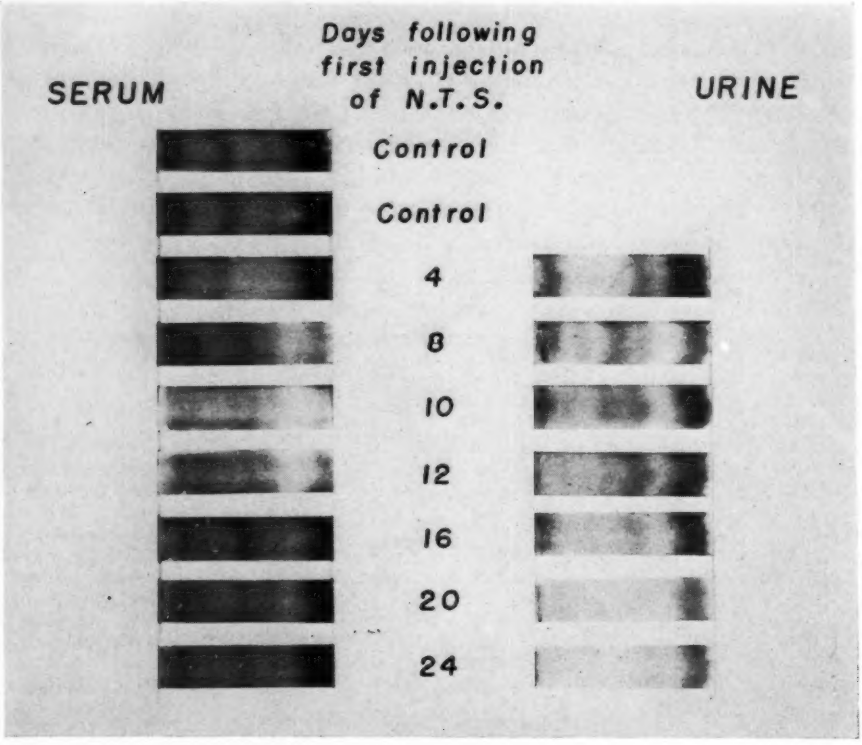


Fig. 2.—Paper electrophoretic patterns of serum and urinary proteins in experimental nephrosis (albumin fraction on the left, gamma-globulin fraction on the right side of each paper strip).

The alpha$_2$-globulin fraction showed the least consistent changes, as is shown by the large standard errors of the means. Nevertheless, there was a marked rise immediately after the injection of NTS and in general the values remained high.

The beta-globulin concentrations showed the same tendencies as the alpha$_2$-globulin fraction, but were consistently higher after 8 days.

The gamma-globulin concentration decreased during the period of injections of NTS or immediately thereafter. Between the eighth and twelfth days there was a transient return to normal, but a minimal value, amounting to a decrease of 32.2 per cent from the control value, was reached a few days later. The electrophoretic patterns of serum and urinary proteins of one animal with nephrosis are shown in Fig. 2.

Serum cholesterol (Fig. 3): The serum cholesterol levels began to rise shortly after the first day of injection of NTS. The mean curve of the cholesterol values reached its peak during the eighth to twelfth days. The mean increase at that time was about 60 per cent above the control level. The highest cholesterol levels found during the period of observation varied between 311

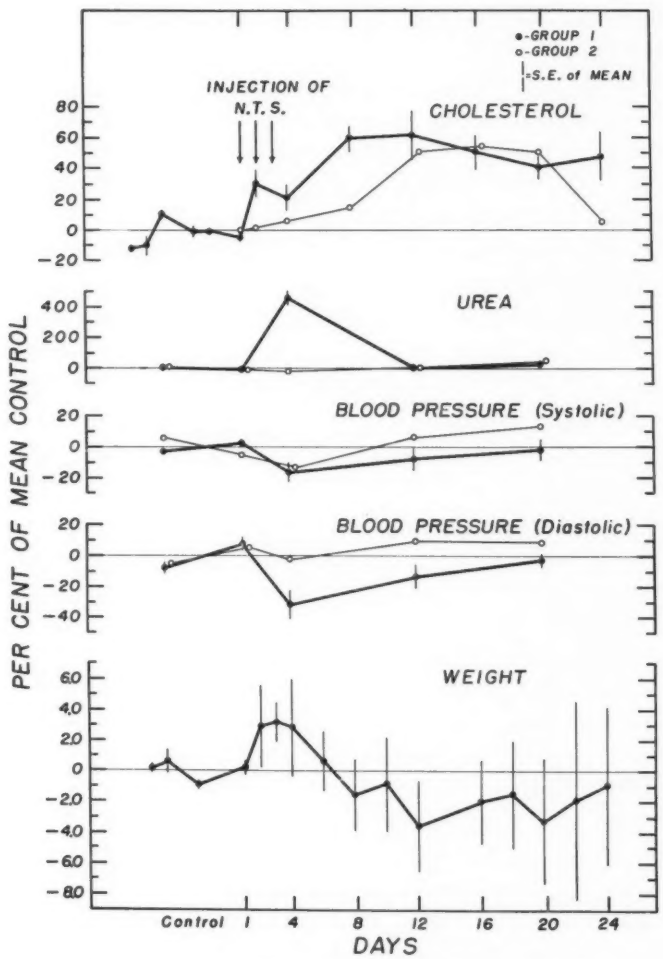


Fig. 3.—Serum cholesterol, blood urea, blood pressure, and weight in experimental nephrosis; changes expressed as per cent of mean control. Group 1 contained nine animals; Group 2 contained four animals.

and 567 mg. per 100 ml. as compared with a normal value of 203 mg. per 100 ml. Normal levels were not found during the period plotted but were, in five animals, found to be within the range of normal after 40 days; in two animals this took 50 to 60 days.

Blood urea (Fig. 3): In four animals the values for blood urea were obtained. A sharp increase in the concentration of urea was observed immediately after the injection of NTS, corresponding to the period of anorexia and vomit-

ing. This increase amounted to 375 to 589 per cent of the mean control value or 118 to 188 mg. per 100 ml. On the twelfth day the values were found to be normal and remained so during the rest of the period of observation.

Blood pressure (Fig. 3): The systolic and diastolic blood pressures fell on the fourth day and returned to normal values toward the end of the period of observation. The decrease was more marked in the diastolic than in the systolic blood pressures (minus 31.5 per cent in the diastolic pressure versus minus 16.4 per cent in the systolic pressure).

Body weight (Fig. 3): The changes in body weight varied considerably. However, there was a definite gain of weight during the first 6 days after the injection of NTS; after this period the mean weight fell to subnormal values. The increase in weight corresponded to the period of oliguria, and the loss of weight coincided with the period of polyuria.

Urinary findings: Five of the nine animals had hemoglobinuria for 2 to 5 days immediately after the injections of NTS. No marked hematuria occurred at any time; on rare occasions 3 to 4 erythrocytes per high power field were observed in the urinary sediment.

Urinary volume (Fig. 4): On the third day of injection of NTS, a reduction of the urinary volume was found; on this and the fourth day the reduction amounted to 23.5 per cent. The daily urine output increased very markedly thereafter and continued to be high until the twentieth day. The maximal average increase was 87 per cent more than the mean control value.

Protein excretion (Fig. 4): The excretion of protein in the urine began on the second day of NTS administration and reached its peak usually on the fourth day. At this time the highest daily protein excretion ranged from 3.97 to 13.5 Gm. The amount of urinary protein decreased after about the fourth day and became negligible between the sixteenth and nineteenth days with the exception of two animals, in which the proteinuria lasted 35 and 38 days, respectively. The protein excretion was highest during the period of oliguria and increase in body weight.

The greater part of the protein in the urine consisted of albumin. This amounted to an average of 58 per cent of the total protein excretion, but it varied between 35.9 and 82 per cent. The variations in the rate of the excretion of globulin fractions were less marked. The $alpha_1$-globulin excretion was relatively high in view of the lowered serum levels of this fraction, and the excretion of beta globulin was not markedly elevated despite the increased serum levels of this fraction. The gamma globulin excretion was relatively low.

Group 2.—The four animals receiving the smaller dose of NTS showed no clinical manifestations of the disease. The mean values of some of the laboratory findings in these animals are plotted in Figs. 1 and 3 as thin lines.

The serum cholesterol levels increased by about 50 per cent, and the reduction of the serum albumin levels was less dramatic than in Group 1. The lowest values of albumin were found between the eighth and the sixteenth days; the

concentration was in general reduced by 37.3 per cent, and the lowest levels ranged from 0.77 to 1.42 Gm. per 100 ml. The globulin concentrations failed to show consistent changes, with the exception of gamma globulin, which showed a definite decrease. The urea concentrations decreased initially but showed some insignificant rise toward the end of the observation period. The blood pressure and the weight did not change significantly.

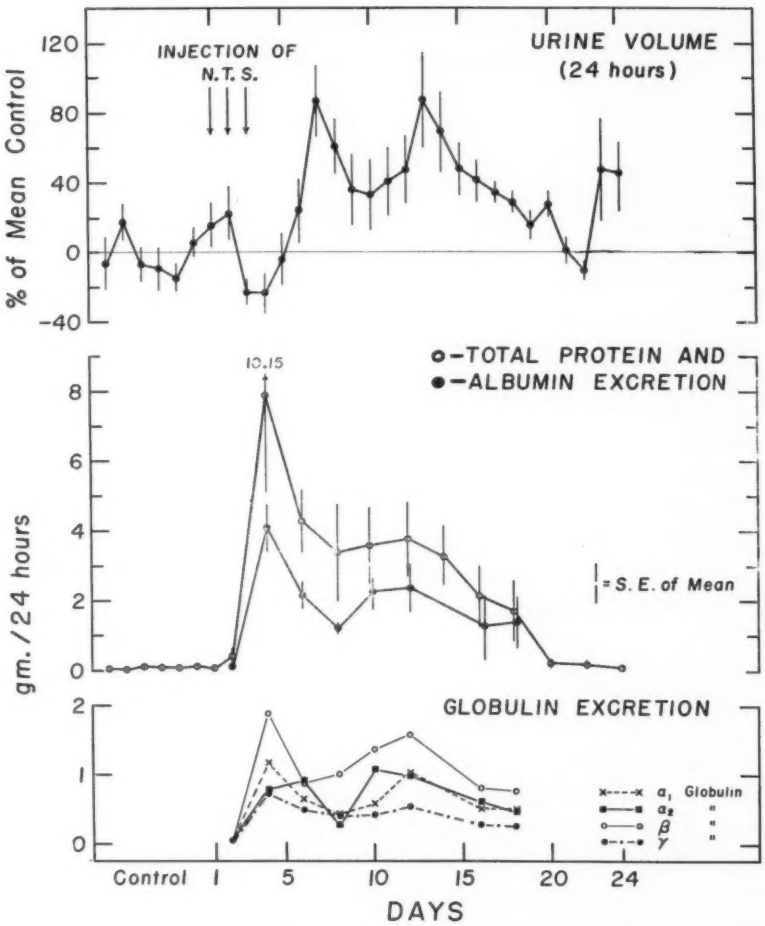


Fig. 4.—Some urinary findings in experimental nephrosis (Group 1, nine animals).

No significant changes were observed in the urine volume until the thirteenth day, when a slight polyuria began amounting to an increase in urine volume of 26 to 85 per cent. This polyuria persisted until the end of the observation period. The proteinuria lasted from the second and fifth days to the sixteenth day. The total protein excretion during the whole period was from 0.6 to 7.2 Gm., the highest daily protein exeretion varying between 0.2 and 4.7 Gm.

CORRELATION BETWEEN SERUM ALBUMIN AND SERUM CHOLESTEROL LEVELS

The curve of the mean concentrations of cholesterol and albumin in the serum appeared to show some inverse relationship. The correlation coefficient between the mean values for cholesterol and albumin of all animals in Group 1 was therefore calculated and found to be minus 0.76 (see also Figs. 1 and 3).

The correlation coefficient for the same two serum components in the two animals with the prolonged course of the disease was found to be minus 0.57 (p less than 0.01) and minus 0.62 (p less than 0.01), respectively.

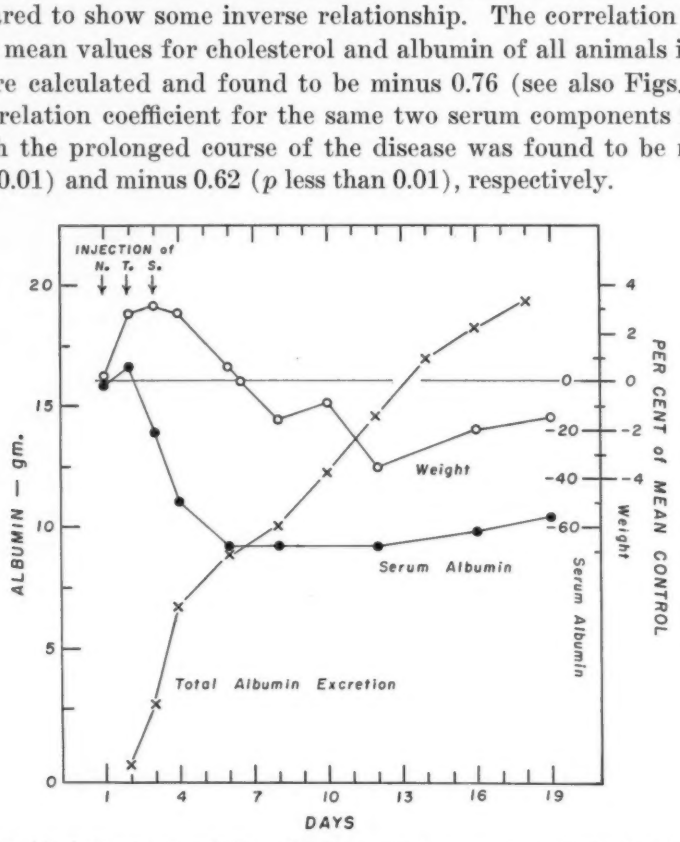


Fig. 5.—Relationship between cumulative albumin excretion, serum albumin levels, and weight in nine nephrotic animals.

RELATION BETWEEN ALBUMIN EXCRETION, ALBUMIN LEVELS IN THE SERUM AND WEIGHT CHANGES IN GROUP 1

In order that the changes of these three variables might be studied more closely, the cumulative albumin excretion, which was obtained by adding the daily average albumin excretion, was plotted with the curves indicating the changes in serum albumin and the weight of the dogs following the injection of NTS (Fig. 5). The slope of the curve of the albumin excretion is initially very steep, indicating a rapid loss of albumin, but flattens out between the fourth and sixth days, yet still showing a substantial daily loss. The decrease in the serum albumin levels shows initially a similar change in its rapidity, but the curve becomes flat after the sixth day, indicating a stable serum level despite the continuous albumin excretion. After a further decrease in the albumin excretion, the albumin levels begin to rise again. The weight increase was present for only 6 days, at which time the albumin levels and albumin excretion seemed to have come into an equilibrium.

COMMENT

The main difficulty encountered in the production of experimental renal disease in dogs was due to variations in the potency of NTS. The preparation first used for two animals in Group 1 produced a prolonged illness which lasted about twice as long as the disease produced by preparations used later. The second sample of NTS led to the most dramatic course of the disease, resulting in the death of one animal fairly soon after the injection, and in the other animal it produced severe edema and death. For the rest of Group 1 (five animals), pooled NTS was used, resulting in a more uniform response, but it failed to produce a disease of very chronic character or a very acute disease resulting in edema and death. The same serum but in smaller amounts had been used in Group 2.

Nevertheless, the disease process showed similar changes in all nine animals of Group 1. All had marked proteinuria, hypoproteinemia, hypoalbuminemia, decrease in the alpha$_1$-globulin and gamma-globulin fractions, and an increase in the concentrations of the alpha$_2$-globulin and beta-globulin fractions. Hypercholesteremia was a consistent finding. Clinical edema was found in one animal only, but the initial increase in the weight of all animals seems to indicate some retention of fluid. Marked hematuria was not observed. Azotemia was only initially present in the four animals in which the blood urea was determined. It was not long before the values returned to normal levels. No hypertension was observed. All these facts show on clinical grounds that the animals presented a picture fitting into the definition of the nephrotic syndrome.[14, 15] The findings are in agreement with those of other authors as far as the electrophoretic changes in experimental nephropathy in other laboratory animals are concerned and with Heymann's findings in puppies after the injection of NTS.

Certain features of the disease in humans, namely, the chronicity of the disease, the mortality rate, the degree of hypercholesteremia, and the increase in the alpha$_2$-globulin and beta-globulin fractions in the serum are different from features of the experimental disease. The differences are quantitative rather than qualitative. Hemoglobinuria, which was encountered in some of the dogs, is absent in human nephrosis. However, it probably was caused by hemolysins present in the NTS.

These deviations are not marked enough to allow the conclusion that the experimental disease produced in animals is principally different from the nephrotic syndrome in humans. On the contrary, the impression is gained that the experimental disease in animals simulates the human disease sufficiently to allow conclusions about the pathogenesis of the nephrotic syndrome, but it may be questioned whether any information about the etiology of the disease can be obtained.

The results in experimental nephrosis seem to indicate that the primary change is the proteinuria. This is probably due to an increased permeability of the glomerular capillaries to protein, as Bell[16] had suggested in human nephrosis, a fact which was proved by Chinard and associates.[17] Randerath[18]

expressed the same impression and thought that the diseased glomerulus becomes more permeable to the smaller protein molecules, primarily albumin. This hyothesis is substantiated by the observations of Jahnke and Scholtan,[19] Slater,[20] Wallenius,[21] and protein fraction clearance[15] experiments. The larger $alpha_2$ and beta globulins are excreted in smaller amounts and, as Kramer[22] and Hardwicke[23] showed, these fractions excreted in the urine did not contain any lipoid material in contrast to the same fractions in the serum. During the course of nephrotoxic nephritis in dogs, Fouts, Corcoran, and Page[24] noted a decrease in glomerular efficiency which was attributed to thickening of the glomerular basement membrane.

The loss of protein, mainly albumin, in the urine causes a rapid fall of the albumin level in the serum; but after a few days, this fall does not continue as rapidly as expected from the steady loss of albumin in the urine. The findings of Drabkin and Marsh[5] in experimental nephrosis in rats and Kramer[22] in human nephrosis support the impression that there is an increased production of albumin taking place, which keeps the serum level and the loss in the urine in balance. Lewis[25] reported a large increase in plasma albumin in normally growing dogs between the ages of 6 and 8 months. In dogs receiving smaller doses of NTS and having therefore less proteinuria, the serum changes are much less marked.

The decrease in serum albumin levels is accompanied by an increase in serum cholesterol. The correlation between these two findings may represent a so-called "empty correlation" but suggests a causal relationship. The observations of other investigators seem to support the latter hypothesis. Fishberg and Fishberg[26] thought first that the hypercholesteremia is secondary to the loss of protein. They could demonstrate in rabbits after bleeding a decrease of the serum albumin levels and an increase in the concentration of cholesterol. Roodenburg[27] reviewed the literature on this subject and added his observations on dogs which he submitted to plasmapheresis. After the serum protein level was lowered, an increase in the serum cholesterol concentrations was noted. This was confirmed by Cleve and Hartmann.[28] Slater,[20] in studying a 25-year-old patient suffering from the nephrotic syndrome over a period of 380 days, found an inverse correlation between the albumin and cholesterol levels during the course of the disease. The albumin levels were low at the outset of the study, while the serum cholesterol levels were high. After corticotropic hormone was given, the albumin levels increased while the cholesterol values were reduced. With a recurrence of the edema, the albumin values became lower again, while the cholesterol values increased. Hardwicke[23] found an inverse linear relationship between the changes in serum albumin and serum cholesterol in 15 patients suffering from the nephrotic syndrome. In addition to that, he observed changes in the electrophoretic patterns similar to what we have found in the experimental disease. The fall in the serum albumin concentrations was accompanied by a fall in the concentrations of $alpha_1$ and gamma globulin, while the concentrations of $alpha_2$ and beta globulin increased.

SUMMARY

Rabbits were sensitized to a suspension of dog kidney. The serum obtained from these rabbits, called nephrotoxic serum (NTS), was administered intravenously in relatively large doses to nine dogs, and in smaller doses to four dogs.

The nine animals receiving larger doses developed proteinuria, which lasted from 16 to 38 days. The main portion of the excreted protein consisted of albumin. Over a period of 40 to 80 days, hypoproteinemia resulted, which was primarily caused by a decrease of the serum albumin. The concentrations of alpha$_1$ globulin and gamma globulin decreased to a lesser extent, while the concentrations of alpha$_2$ globulin and beta globulin increased. Prolonged hypercholesteremia was a persistent finding. Azotemia was transient. The blood pressures fell initially but returned to normal values toward the end of the period of observation. In all animals there was initial retention of fluid during a period of oliguria, but the edema disappeared during a period of polyuria. Only one animal developed marked edema. Two animals in this series died, all other animals recovered completely. Normal serum protein values were usually seen within 40 days, but in two animals not until 80 days.

The animals receiving smaller doses of NTS had a proportionately less severe disease with less proteinuria and less marked hypoproteinemia and hypercholesteremia.

The relationship between the amount of albuminuria and the resulting serum albumin levels is discussed. An inverse relationship between the albumin and the cholesterol levels in the serum was found during experimental renal disease.

REFERENCES

1. Heymann, Walter: III. Nephrotic Syndrome Induced by Injection of Anti-kidney Serum, Methods in Medical Research, Vol. 5, Chicago, 1952, The Year Book Publishers, Inc., pp. 264-267.
2. Heymann, W.: The Experimental Nephrotic Syndrome in Animals, *in* Proceedings of the Fifth Annual Conference on the Nephrotic Syndrome, New York, 1954, National Nephrosis Foundation, Inc.
3. Hieronymi, G., Bohle, A., and Hartmann, F.: Morphologische und elektrophoretische Untersuchungen bei der Masuginephritis an Ratten, Arch. Kreislaufforsch. **18**: 34-49, 1952.
4. Moench, A., Rother, C., Sarre, H. J., and Sartorius, H.: Die nephrotische Komponente im Ablauf der experimentellen Nephritis nach MASUGI, Verhandl. deutsch. Gesellsch. inn. Med. **59**: 458-468, 1953.
5. Drabkin, D. L., and Marsh, J. B.: Metabolic Channeling in Experimental Nephrosis. I. Protein and Carbohydrate Metabolism, J. Biol. Chem. **212**: 623-631, 1955.
6. McCrory, W.: The Application of Paper Electrophoresis to Animal and Human Material. Proceedings of the Fifth Annual Conference on the Nephrotic Syndrome, New York, 1954, National Nephrosis Foundation, Inc.
7. Kingsley, G. R.: The Direct Biuret Method for the Determination of Serum Proteins as Applied to Photoelectric and Visual Colorimetry, J. LAB. & CLIN. MED. **27**: 840-845, 1941-1942.
8. Durrum, E. L.: A Microelectrophoretic and Microionophoretic Technique, Am. Chem. Soc. **72**: 2943-2948, 1950.
9. Grassmann, W., Hannig, K., and Knedel, M.: Über ein Verfahren zur elektrophoretischen Bestimmung der Serumproteine auf Filtrierpapier, Deutsche med. Wchnschr. **76**: 333-336, 1951.
10. Zlatkis, Albert, Zak, Bennie, and Boyle, A. J.: A New Method for the Direct Determination of Serum Cholesterol, J. LAB. & CLIN. MED. **41**: 486-492, 1953.

11. Van Slyke, D. D., and Cullen, G. E.: The Determination of Urea by the Urease Method, J. Biol. Chem. **24**: 117-122, 1916.
12. Sobel, A. E., Yuska, Henry, and Cohen, Julius: A Convenient Method of Determining Small Amounts of Ammonia and Other Bases by the Use of Boric Acid, J. Biol. Chem. **118**: 443-446, 1937.
13. Wood, E. H.: Special Instrumentation Problems Encountered in Physiological Research Concerning the Heart and Circulation in Man, Science **112**: 707-715, 1950.
14. Stickler, G. B., Burke, E. C., and McKenzie, B. F.: Electrophoretic Studies of the Nephrotic Syndrome in Children: Preliminary Report, Proc. Staff Meet., Mayo Clin. **29**: 555-561, 1954.
15. Stickler, G. B., McKenzie, B. F., Wakim, K. G., and Burke, E. C.: The Effect of Plasma Transfusion and Treatment With Corticotropin on the Electrophoretic Patterns in Serum and Urine of Children With the Nephrotic Syndrome, J. LAB. & CLIN. MED. **47**: 392-402, 1956.
16. Bell, E. T.: Textbook of Pathology, ed. 6, Philadelphia, 1947, Lea and Febiger, pp. 910.
17. Chinard, F. P., Lauson, H. D., Eder, H. A., Greif, R. L., and Hiller, A.: A Study of the Mechanism of Proteinuria in Patients With the Nephrotic Syndrome, J. Clin. Invest. **33**: 621-628, 1954.
18. Randerath, E.: Nephrose-Nephritis, Klin. Wchnschr. **20**: 281-284, 1941.
19. Jahnke, K., and Scholtan, W.: Zum Mechanismus der Proteinurie, Deutsches Arch. klin. Med. **200**: 821-837, 1953.
20. Slater, R. J.: Discussion, *in* Proceedings of the Fifth Annual Conference on the Nephrotic Syndrome, New York, 1954, National Nephrosis Foundation, Inc., p. 79.
21. Wallenius, Gunnar: Renal Clearance of Dextran as a Measure of Glomerular Permeability, Acta Soc. med. upsalien. **59**(suppl. 4): 1-91, 1954.
22. Kramer, Benjamin: *in* Proceedings of the Fifth Annual Conference on the Nephrotic Syndrome, New York, 1954, National Nephrosis Foundation, Inc., p. 161.
23. Hardwicke, J.: Serum and Urinary Protein Changes in the Nephrotic Syndrome, Proc. Roy. Soc. Med. **47**: 832-834, 1954.
24. Fouts, P. J., Corcoran, A. C., and Page, I. H.: Observations on the Clinical and Functional Course of Nephrotoxic Nephritis in Dogs, Am. J. M. Sc. **201**: 313-326, 1941.
25. Lewis, L. A.: Changes That Occur in Plasma Proteins During Growth of the Dog, J. Biol. Chem. **162**: 473-476, 1946.
26. Fishberg, Ella H., and Fishberg, A. M.: The Mechanism of the Lipemia of Bleeding, Proc. Soc. Exper. Biol. & Med. **25**: 296-299, 1927-1928.
27. Roodenburg, Andries: The Reaction of Plasma Lipoids to Plasmapheresis in Thyroidectomized Dogs, Proc. Staff Meet., Mayo Clin. **20**: 6-12, 1945.
28. Cleve, Hartwig, and Hartmann, Fritz: Zur Entstehung von Nephrosen durch Plasmapherese am Hund, Arch. exper. Path. u. Pharmakol. **223**: 198-202, 1954.

QUANTITATIVE COMPARISON OF COUGH-SUPPRESSING EFFECTS OF ROMILAR AND OTHER ANTITUSSIVES

LEO J. CASS, M.D.,* AND WILLEM S. FREDERIK, PH.D., M.D.**
BOSTON, MASS.

IT WAS demonstrated in previous papers[1, 2] that the antitussive effectiveness of dextromethorphan† hydrobromide‡ and of codeine are of the same order of magnitude. Ralph[3] reported that dextromethorphan is twice as active as codeine in suppressing cough but stated that his figures "are an approximation rather than a mathematical comparison." Both groups of workers are agreed that dextromethorphan does not produce the side effects typical of codeine.

It seemed of interest to re-evaluate the comparative activity of dextromethorphan and codeine and to include in the study caramiphen ethanedisulfonate‡§ which, as dextromethorphan, has the advantage of not being addiction liable.

METHOD

The plan was to compare two dose levels of dextromethorphan (10 and 20 mg.), 15 mg. of codeine sulfate,‡ 10 mg. of caramiphen ethanedisulfonate, and placebo medication. The five materials were supplied in tablets of identical appearance and were delivered under the following code designations: "Q," "R," "S," "T," and "U." The key of the code remained unknown to the medical and nursing staffs throughout the study.‖

One hundred twenty patients with persistent cough were selected from the population of Long Island Hospital in Boston. All subjects received the five medications for a period of ten days each. The administration was in a random sequence determined by chance selection. Moreover, to avoid carry-over effects of the materials to be tested, each patient was given placebo tablets‖¶ for 3 days between each of the 10-day periods of administration of coded medications. In addition, all patients received placebo tablets for 3 days terminally so that a comparison could be made of the cough intensity during each of the five 3-day periods following the 10-day medication periods. Thus, the total experimental period was 65 days. All tablets were given q.i.d.—namely, at 7 A.M., 11 A.M., 3 P.M., and 7 P.M.

From the Department of Hygiene, Harvard University, Cambridge, Mass. and the Department of Physiology, Harvard School of Public Health, Boston, Mass.

Received for publication May 24, 1956.

*Physician, University Health Services, Harvard University, Cambridge, Mass.; Visiting Physician, Long Island Hospital, Boston, Mass.

**Research Associate, Department of Physiology, Harvard School of Public Health.

†Dextromethorphan is the generic name of dextro-3-methoxy-N-methylmorphinan (Romilar).

‡For brevity, dextromethorphan hydrobromide, caramiphen ethanedisulfonate, and codeine sulfate will usually be referred to hereafter as dextromethorphan, caramiphen, and codeine, respectively.

§Caramiphen ethanedisulphonate is the generic designation of bis-(1-[carbo-β-diethylaminoethoxy]-1-phenylcyclopentane)-ethanedisulphonate (Toryn).

‖All medications for this investigation were supplied by Dr. Leo A. Pirk of Hoffmann-La Roche Inc., Nutley, N. J.

¶The identity of these placebo tablets which simulated the five test materials in shape, size, and color was known to us, but not to the observer or the patients.

Fifty-seven patients had to be excluded in the course of the study either because of interfering disease, objections on the part of the patient to serving as experimental subject, or because of difficulties on the part of the observer in rating the degree of cough. Thus, 63 patients actually completed the experiment according to plan.

All observations were made by a single observer. As in our previous studies[1, 2, 4] the method of observation involved a scale of arbitrary values. For recording the data, the degree of cough was rated numerically from 0 to 4: 0 indicated no cough; 1, occasional, barely troublesome cough; 2, moderately troublesome cough; 3, markedly troublesome cough; and 4, incessant and distressing cough. Since three observations (at 8 A.M., 12 noon, and 4 P.M.) were made for every patient daily, the number of observations used in the final analysis amounted to 63 (patients) × 3 (observations per day) × 65 (days of experimental period), or 12,285 observations.

The scale was sliding and relative for each patient so that only the change in degree of cough was important. By aggregating all recorded observations and by reducing them to simplest terms, the findings could be summarized in tabular and graphic form. Thus, Table I lists the average degrees of cough determined from the ratings recorded for all 63 patients during each of the five periods when they received the unknown medications, as calculated *A*, for each of the three daily observations during the 10-day periods; *B*, for all three daily observations on each of the 10 days of administration; *C*, for all observations. Furthermore, this table lists the average degrees of cough determined from the ratings recorded for all 63 patients during each of the five periods when they received "known" placebo medication, as calculated *D*, for all three daily observations on each of the three days of administration following the 10-day periods of unknown medication; and *E*, for all observations.

RESULTS

As is seen, the coded drugs are arranged in this table according to their effectiveness, as judged from their over-all performance (see column *c*).

Fig. 1, which corresponds to section *a* of Table I, presents graphically the average daily variations of degree of cough of the 63 patients at the three periods of observation when they received the five unknown medications.

Fig. 2, which corresponds to sections *b* and *d* of Table I, presents graphically the average variations of degree of cough of the 63 patients on each of the 10 days of administration of the five unknown medications and on each of the three days of administration of "known" placebo tablets following the periods of unknown medications.

Fig. 3 presents the straight lines fitted to the logarithms of the numerical values of the average degrees of cough on the 10 days each on which the five unknown medications were administered. The fit is, in general, satisfactory.

It is evident from Table I and from Figs. 1, 2, and 3 that "Q" has minimal antitussive activity, if any, and that "R" has the greatest effectiveness. From Fig. 1 it appears that, with all medications, the degree of cough is maximal in the morning, minimal at noon, and intermediate in the afternoon. If "Q" is the "unknown" placebo medication, then the curve for "Q" in Fig. 1 represents the daily variations of degree of cough of our patients when they did not receive antitussive medication. As is seen from this figure, the sequence of the relative antitussive effectiveness of the four active drugs remained the same during the

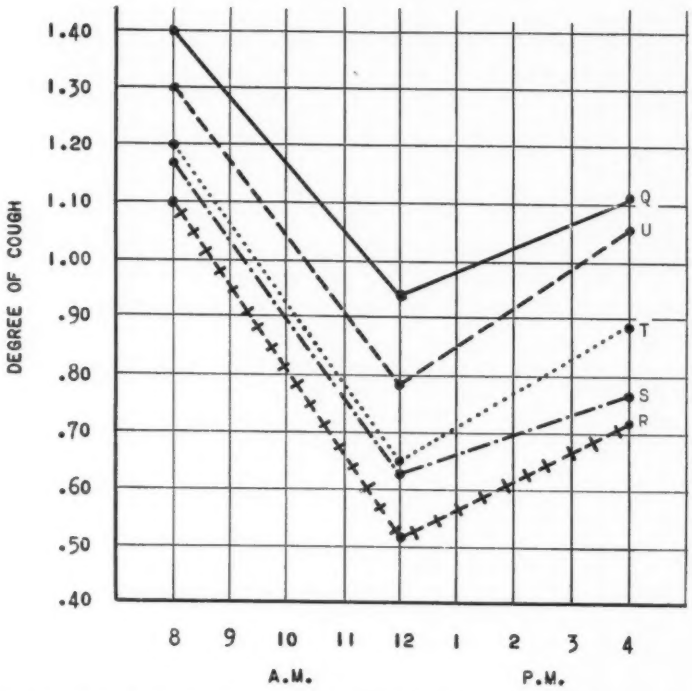


Fig. 1.—Average daily variations of degree of cough of 63 patients at 8 A.M., 12 noon, and 4 P.M. who received five unknown medications ("Q," "R," "S," "T" and "U") for ten days each.

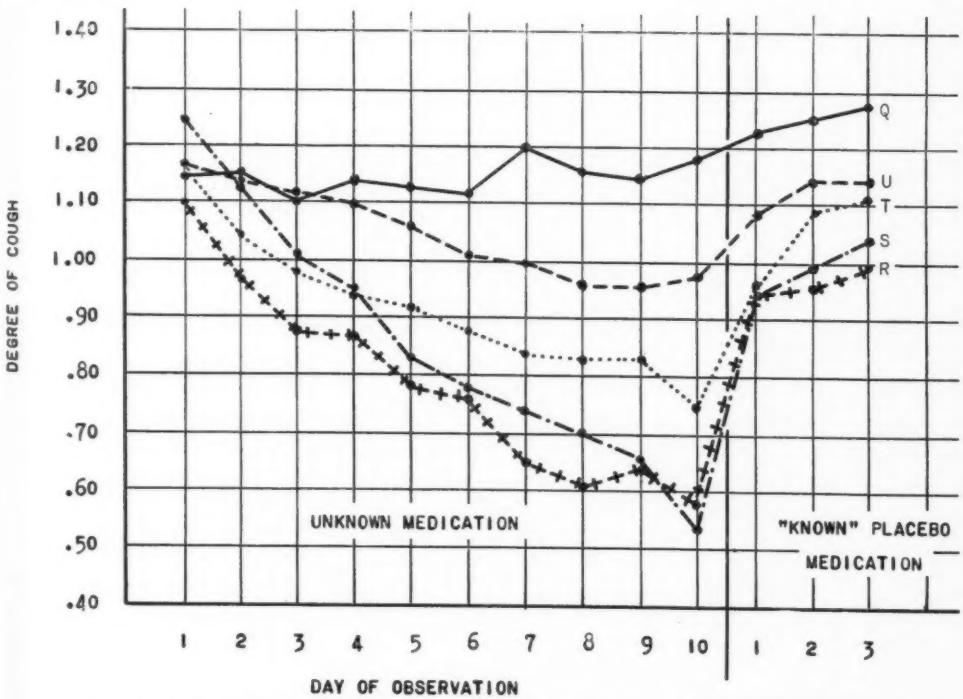


Fig. 2.—Average variations of degree of cough of 63 patients who received five unknown medications ("Q," "R," "S," "T" and "U") for ten days each and "known" placebo tablets for three days each following the periods of unknown medications.

TABLE I. AVERAGES OF DEGREE OF COUGH

CODE OF UNKNOWN MEDICATION	A. AVERAGES* OF ALL OBSERVATIONS WITH EACH OF THE FIVE UNKNOWN MEDICATIONS, AT			B. AVERAGES† OF ALL OBSERVATIONS WITH THE FIVE UNKNOWN MEDICATIONS ON EACH OF THE TEN DAYS OF ADMINISTRATION										C. AVERAGES‡ OF "B" VALUES SUB	D. AVERAGES§ OF ALL OBSERVATIONS ON EACH OF THE 3 DAYS OF ADMINISTRATION OF "KNOWN" PLACEBO TABLETS FOLLOWING PERIODS OF UNKNOWN MEDICATION			E. AVERAGES‖ OF "D" VALUES SUB
	8 A.M.	12 NOON	4 P.M.	1	2	3	4	5	6	7	8	9	10		1	2	3	
Q	1.40	0.94	1.11	1.15	1.15	1.11	1.14	1.13	1.12	1.20	1.16	1.15	1.18	1.15	1.23	1.25	1.28	1.25
U	1.31	0.78	1.06	1.17	1.14	1.12	1.10	1.06	1.01	1.00	0.96	0.96	0.97	1.05	1.10	1.14	1.14	1.13
T	1.21	0.65	0.89	1.16	1.04	0.98	0.94	0.92	0.88	0.84	0.83	0.83	0.75	0.92	0.96	1.09	1.11	1.05
S	1.18	0.63	0.77	1.25	1.13	1.01	0.95	0.83	0.78	0.74	0.70	0.66	0.54	0.86	0.94	0.99	1.04	0.99
R	1.11	0.52	0.72	1.10	0.97	0.88	0.87	0.78	0.76	0.65	0.61	0.64	0.58	0.78	0.94	0.96	1.00	0.97

Sixty-three patients who received five unknown medications (Q, R, S, T, and U) for ten days each with three daily observations and "known" placebo medication for three days each following the ten-day periods of unknown medication.

*Each figure represents average of 630 observations.
†Each figure represents average of 189 observations
‡Each figure represents average of 1,890 observations.
§Each figure represents average of 189 observations.
‖Each figure represents average of 567 observations.

day. By the same token, if, for example, compound "U" provided greater cough-suppressing activity than compound "T" in the morning, this was also true at noon and in the afternoon.

As is seen from Fig. 2, the ineffectiveness of "Q" does not change considerably during the 10-day period of administration, and this also suggests that "Q" is the placebo medication. With the four other unknown materials, there occurred almost invariably a daily increase of antitussive effectiveness. This might be due to cumulative action of the drugs administered, but it may also be interpreted as interruption of the vicious circle—cough-creating irritation of the respiratory tract mucosa, which, in turn, gives rise to another cough—by antitussive medication leading to more marked cough-suppressing effects day by day.

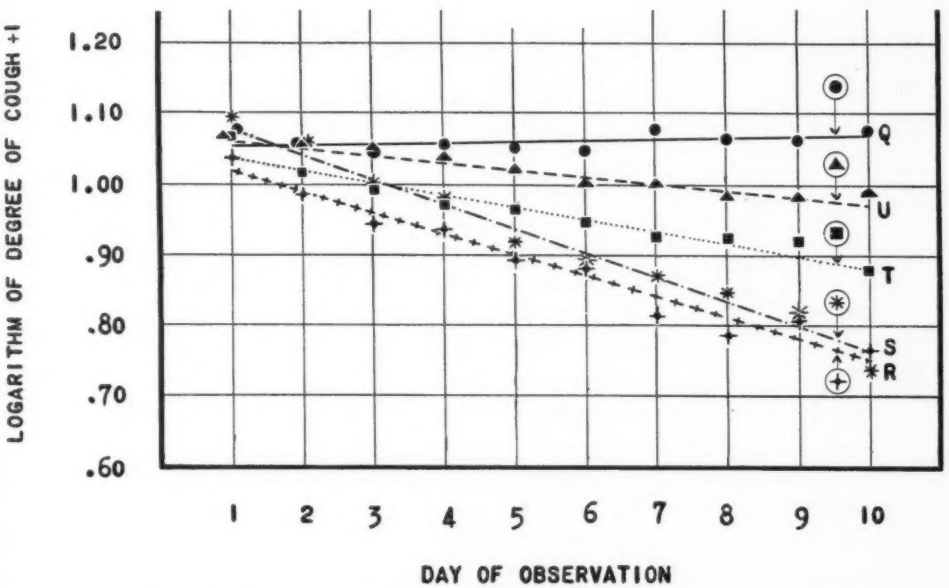


Fig. 3.—Straight lines fitted to logarithms of average degrees of cough of 63 patients who received five unknown medications ("Q," "R," "S," "T," and "U") for ten days each.

If one compares the average degrees of cough on the tenth days of administration of the five unknown medications and on the corresponding first days of administration of "known" placebo tablets, it appears that the change from "Q" to "known" placebo tablets is attended by a 4 per cent increase of degree of cough and that the changes from "U," "T," "S," and "R" to "known" placebo tablets is associated with 13 per cent, 28 per cent, 74 per cent, and 62 per cent increases of degree of cough, respectively. This again suggests that "Q" is the unknown placebo medication and that "U," "T," "S," and "R" are the active materials.

If a similar comparison is made between the over-all averages of degree of cough on the 10 days of administration of each of the 5 unknown medica-

tions and the corresponding over-all averages of degree of cough on the three days each of administration of "known" placebo tablets, it is seen that the changes were attended by the following increases of degree of cough:

	PER CENT INCREASE OF DEGREE OF COUGH
Change from "Q" to "known" placebo tablets	8
Change from "U" to "known" placebo tablets	7
Change from "T" to "known" placebo tablets	14
Change from "S" to "known" placebo tablets	15
Change from "R" to "known" placebo tablets	24

These figures suggest that "U" was less effective in suppressing cough than "T," "S," and "R" and that "R" had greater antitussive activity than "T" and "S."

It was tentatively concluded that "Q" was the unknown placebo medication and that "R" was the most effective antitussive agent. Although the code was broken only after statistical evaluation of our findings, the designations of the five materials, arranged according to their relative effectiveness, are identified below for better understanding.

CODE DESIGNATION	EFFECTIVENESS	IDENTIFICATION OF DRUG
"Q"	Least effective	Placebo
"U"		Caramiphen ethanedisulfonate, 10 mg.
"T"		Dextromethorphan hydrobromide, 20 mg.
"S"		Dextromethorphan hydrobromide, 10 mg.
"R"	Most effective	Codeine sulfate, 15 mg.

With the purpose of investigating the real significance of the findings presented above, the data were subjected to a statistical analysis.

It was found that dextromethorphan (10 mg. and 20 mg.), codeine (15 mg.), and caramiphen (10 mg.) were all significantly more effective in suppressing cough than the unknown placebo medication ($p < 0.01$).

Furthermore, it appeared that dextromethorphan (10 mg. and 20 mg.) and codeine (15 mg.) all had significantly greater antitussive activity than caramiphen ($p < 0.01$).

Finally, it was found that the cough-suppressing effects of dextromethorphan at the two-dose levels (20 mg. and 10 mg.) on the one hand and of dextromethorphan (10 mg.) and codeine (15 mg.) on the other were not significantly different ($p > 0.01$).

SUMMARY AND CONCLUSIONS

A "double blind" study was carried out to compare the antitussive effectiveness of dextromethorphan hydrobromide at two-dose levels (10 mg. and 20 mg.), codeine sulfate (15 mg.), caramiphen ethanedisulfonate (10 mg.), and of placebo medication. The five materials were supplied as tablets of

identical appearance under code designations. All medications were given four times a day for 10 days each. Each such period of drug administration was followed by three days on which the patients received "known" placebo medication four times a day.

Sixty-three subjects with chronic cough completed the program. They received the materials to be tested in a random sequence. The cough-suppressing activity of the drugs and the "known" placebo tablets was recorded three times daily by means of a numerical scale.

After aggregating the more than 12,000 observations, the five coded drugs were ranked in order of their antitussive effectiveness. The material which had the least cough-suppressing activity was thought to be the placebo, and this was confirmed when the drugs were decoded after mathematical treatment of all data. The statistical analysis revealed that dextromethorphan hydrobromide at both dose levels, codeine sulfate, and caramiphen ethanedisulfonate were all significantly more effective than the placebo medication; that dextromethorphan hydrobromide at both dose levels and codeine sulfate had significantly greater activity than caramiphen ethanedisulfonate; and that the effects of the two-dose levels of dextromethorphan hydrobromide and of the lower dose level of this compound and of codeine sulfate were not significantly different.

The following conclusions were drawn:

1. Ten milligram and 20 mg. doses of dextromethorphan (Romilar) hydrobromide, 15 mg. of codeine sulfate, and 10 mg. of caramiphen ethanedisulfonate (Toryn) all have antitussive properties.

2. Ten milligrams of caramiphen ethanedisulfonate is less effective in suppressing cough than 10 mg. or 20 mg. of dextromethorphan hydrobromide or 15 mg. of codeine sulfate.

3. Our method did not differentiate between the degree of antitussive activity of dextromethorphan hydrobromide (10 mg. or 20 mg.) and of codeine sulfate (15 mg.).

4. For all practical purposes dextromethorphan hydrobromide and codeine sulfate may be considered of equal antitussive effectiveness on a weight basis.

REFERENCES

1. Cass, L. J., and Frederik, W. S.: Evaluation of a New Antitussive Agent, New England J. Med. **249**: 132-136, 1953.
2. Cass, L. J., Frederik, W. S., and Andosca, J. B.: Quantitative Comparison of Dextromethorphan Hydrobromide and Codeine, Am. J. M. Sc. **227**: 291-296, 1954.
3. Ralph, N.: Evaluation of a New Cough Suppressant, Am. J. M. Sc. **227**: 297-303, 1954.
4. Cass, L. J., and Frederik, W. S.: Comparative Clinical Effectiveness of Cough Medication, Am. Pract. & Digest Treat. **2**: 844-852, 1951.

(1101 Beacon Street, Brookline, Mass.)

THE RADIOISOTOPE RENOGRAM

An External Test for Individual Kidney Function and Upper Urinary Tract Patency

George V. Taplin, M.D., Orsell M. Meredith, Jr., Ph.D., Harold Kade, M.D., and Chester C. Winter, M.D.
Los Angeles, Calif.

With the Technical Assistance of Patricia Peel and C. William Beckner

INTRODUCTION

PREVIOUS application of external gamma-ray scintillation counting and recording techniques for estimating liver vascularity, hepatic cell function, and biliary tract patency with I^{131}-labeled Rose Bengal[1] suggested that analogous functions of the kidneys might be tested in the same manner. Such an external test using Diodrast I^{131} would permit simultaneous evaluation of three important dynamic functions of the kidneys individually by merely inspecting the patterns of the recorded curves.

Since no similar radioisotope techniques had been described previously, extensive studies were made in animals to develop clinically feasible and safe test procedures. After demonstrating that it was possible to make external measurements with use of tracer doses (10-20 μc) of either I^{131}-tagged Urokon or Diodrast which reflected changes in renal vascularity, tubular cell function, and ureteral patency, we designed further experiments to simulate various clinical and pathologic states. From the results of these laboratory investigations and from previous clinical experience with the radioactive Rose-Bengal liver function test procedure, it was predicted that similar results could be obtained in patients with the use of either I^{131}-tagged Urokon or Diodrast.

The renal uptake and excretion of intravenously injected I^{131}-tagged Diodrast (the Diodrast Renogram), as determined by external gamma-ray scintillation counting techniques, has been developed into a rapid, efficient, clinical test for simultaneously estimating the vascular capacity, tubular cell function of each kidney individually, and the patency of the upper urinary passages. This tracer test provides clinical data of a functional nature which is otherwise obtainable only by excretory urography, aortography, or bilateral ureteral catheterization, as well as being a research tool in further investigations in renal physiology on a dynamic basis.

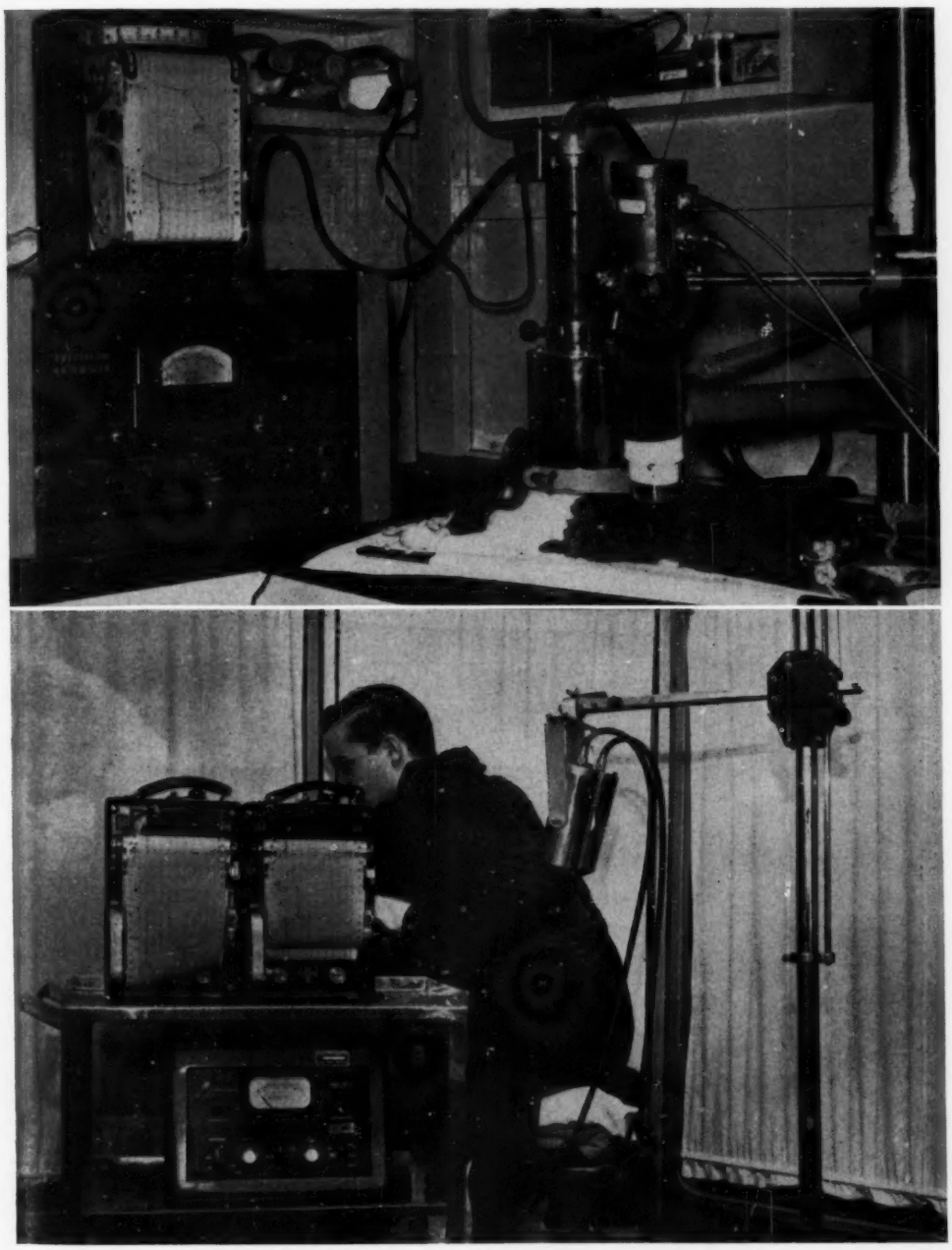
From the Division of Pharmacology and Toxicology, Atomic Energy Project, and the Department of Medicine, School of Medicine, University of California at Los Angeles, and the Medical and Urological Services, Veterans Administration Center, Los Angeles, Calif.

This article is based on work performed under Contract No. AT-04-1-GEN-12 between the Atomic Energy Commission and the University of California at Los Angeles.

Received for publication May 28, 1956.

MATERIALS AND EQUIPMENT

Urokon (sodium 3-acetylamino 2,4,6-triiodobenzoate) was rendered radioactive by an ion exchange reaction with NaI^{131}, yielding a specific activity of approximately 0.10 mc. per milligram. Diodrast (the diethanolamine salt of 3,5 diiodo-4-pyridone-N-acetic acid) was



1a.

1b.

Fig. 1.—*a*, Equipment for performing the Diodrast Renogram and blood clearance test externally in rabbits. *b*, Radiation counting and recording equipment and positioning of scintillation detector heads used clinically in performing the Diodrast Renogram.

tagged with I^{131} by a more complicated synthesis procedure yielding a product with a specific activity of approximately 0.70 mc. per milligram. Rose Bengal labeled with I^{131} was supplied in sterile solution of relatively high specific activity. The commercially available preparations,* initially containing 0.100 to 0.500 mc. per milliliter, were diluted with saline to the desired concentration of radioactivity for intravenous administration.

Three gamma-ray scintillation detectors with thallium-activated sodium iodide crystals were employed. Each was connected through a ratemeter to an Esterline-Angus recorder. The detector units were housed in specially adapted lead shields with adjustable clamp mountings to achieve collimation and to reduce background radioactivity. A deep-well scintillation counter was used to measure the gamma-ray activity in blood, urine, and excised tissue samples.

Technique for Performing the Radioisotope Renogram in Rabbits.—For each test, a virgin female Dutch rabbit (weighing from 1.5 to 2.0 kilograms) was lightly anesthetized with pentobarbital sodium and secured in a prone position on an animal board. A scintillation detector was positioned over each kidney (located by palpation) so as to minimize the recording of extraneous radioactivity coming from other places in the body (Fig. 1*a*) and was maintained in exactly the same position throughout the test. A third scintillation probe, directed at the upper thorax, was used to record changing blood levels of gamma radioactivity.

After the starting of the three chart drives and the recording of the background counting rate from each detector, the test dose of 0.2 to 1.0 ml. (containing 20μc of radioactivity and 0.2 to 8 mg. of Diodrast or Urokon) was injected into a marginal ear vein. These variations in the amounts of carrier injected gave insignificant differences in the dynamics of their renal excretion. The records of counting rate versus time, for each detector, were allowed to run for various periods, approximately 30 minutes.

EXPERIMENTAL RESULTS

Typical Normal Kidney Uptake-Excretion and Blood Clearance Curves.—Fig. 2*a* is a composite of the tracings of gamma-ray activity in the kidneys and blood following the intravenous administration of radioactive Urokon. The radioactivity in the blood (detected over the chest) rises abruptly after intravenous injection and is followed almost immediately by a precipitous (exponential-like) decline. This falling blood level coincides with a steep rise in kidney radioactivity beyond the initial blood dilution level. As the concentration in the kidney increases, the blood level declines to a minimum plateau. In approximately five minutes, the kidney activity curve reaches a momentary maximum which then declines gradually to a plateau in about 10 to 15 minutes. The excretion segment of the renal curve is similar to (but not as rapid as) the decline in the blood level.

This sequence of events shows the dynamic relationships in the uptake and excretion of the tracer. The first initial upsurge (segment *A* in Fig. 2*a*) of radioactivity in the kidney area is synchronous with dispersion of isotope throughout the circulation following intravenous injection and merely reflects the presence of radioactivity in the vascular bed of the organ and surrounding tissues. The secondary rise in radioactivity over the renal area (segment *B*) is associated with a reciprocal decline in blood level. This relationship indicates that segment *B* of the renal curve represents an active secretory process in the kidneys. The rate of accumulation of isotope in the kidney then decreases since progressively lesser amounts of radioactive material remain available for uptake as clearance from the blood stream continues. When this rate of kidney

*Radioisotope Division of Abbott Laboratories, Oak Ridge, Tenn.

accumulation is equaled by the progressively increasing gradient of excretion, the level of radioactivity within the kidney is at a transitory equilibrium of maximum contents. As the rate of urinary excretion exceeds that of renal uptake from the blood, the process of clearance from the kidney areas becomes manifest (segment *C*).

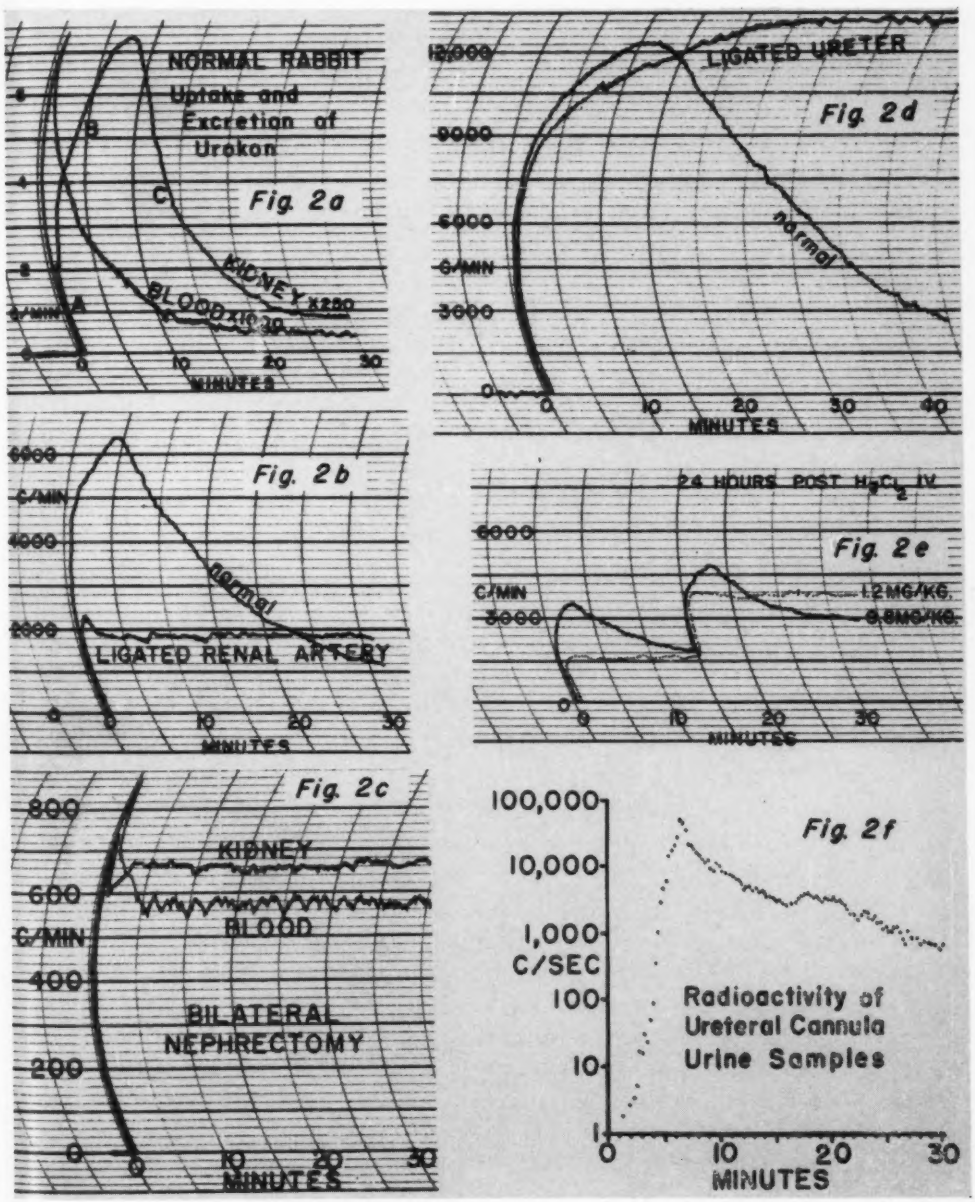


Fig. 2.—Blood clearance and renal uptake-excretion curves obtained in normal rabbits and in animals with various renal-urteral abnormalities. *a*, This figure is the key to all subsequent illustrations in this report in respect to designation of the three main segments labeled *A*, *B*, and *C* of the Diodrast Renograms. It also demonstrates the reciprocal time relationship between blood clearance and active tubular cell secretion of I^{131}-labeled Diodrast (segment *B*).

Series of specially designed experiments were performed in groups of two to six rabbits each to establish the significance of each of the three major segments of the radioisotope renogram individually, in order to anticipate and categorize the abnormalities which might be observed in the application of this test to patients.

Ligation of Renal Artery.—The effect of a totally infarcted or an absent kidney was simulated by unilateral ligation of the renal artery (Fig. 2*b*). No active localization of radioactivity occurred over this kidney (absent segments *B* and *C*). Only the appearance of isotope in the general circulation is recorded.

Bilateral Nephrectomy.—The pattern of the renogram in these circumstances (Fig. 2*c*) shows only the vascular dispersion of radioactivity in the kidney areas with no selective localization of isotope. In addition, the blood level of gamma-ray activity does not fall rapidly from its peak in the absence of kidney function.

With elevated blood levels of radioactive Urokon (due to total absence of kidney function) the possible localization of I^{131} in the thyroid required investigation; therefore, a second 20 μc dose of Urokon was injected intravenously and the animal was sacrificed after three hours. The radioactivity levels of excised tissue samples, as assayed in the deep-well gamma scintillation counter, showed no significant localization of activity in the thyroid or liver. The total activity contained in the thyroid gland was only 6 mμc, whereas the blood contained 60 mμc per milliliter.

Ligation of Ureter.—The effect of complete ureteral obstruction (from calculus, stricture, or tumor) was simulated by surgical ligation of one ureter (Fig. 2*d*). On the obstructed side, segment *B* of the curve continues beyond the period when the curve over the normal kidney shows almost complete clearance. This evidence indicates that in acute ureteral obstruction the tracer substance, once actively secreted by the kidney tubules, is *not* rapidly reabsorbed into the circulation to be excreted by the other kidney or by some other organ.

Mercuric Chloride Poisoning.—In simulating nephrosis, renal tubular damage was produced by intravenous administration of mercuric chloride at various dose levels in the range from 2 to 2,000 μg per kilogram. Individual animals tested 24 hours later with both Urokon and Diodrast, given in sequence, showed graded depression of excretory function (Fig. 2*e*). One group of animals was followed with serial determinations of kidney function. When the rabbits were sacrificed, histologic sections were prepared which showed changes typical of mercuric chloride poisoning varying from "cloudy swelling" and early necrosis of tubular epithelium to total excretory obstruction by inspissated casts of proteinaceous detritus completely occluding the distal convoluted and collecting tubules. The severity of microscopic changes observed corresponded fairly well to both the size of the dose of mercuric chloride and to the degree of impairment indicated by the radioisotope renograms.

Ureteral Cannulation.—In order that the rate of disappearance of radioactivity from the kidney area as indicated by external gamma scintillation

recording might be evaluated, the right ureter of a normal animal was transected, the distal end ligated, and the proximal end cannulated. During a 30-minute period following intravenous injection of radioactive Diodrast, 120 serial samples of urine were collected on filter papers from the open end of the cannula, with each sample representing the urinary output of that kidney for a 15-second interval. The radioactivity in each sample of urine was counted individually, and the results were plotted on 5-cycle semilogarithm paper (Fig. 2*f*).

The curve representing the actual excretion rate demonstrates that Diodrast in trace doses is handled by an active secretory process because the curve remains practically a true exponential function for the entire period except in its initial phase. (The 4-minute delay in appearance of radioactivity can be attributed to the length and volume of the polyethylene tube and to streaming effects.)

With use of rabbits with similarly cannulated ureters, a series of experiments was performed measuring dropwise excretion of urinary radioactivity and simultaneously recording the dynamic changes in renal radioactivity levels and circulating blood levels of radioactivity by external gamma scintillation detectors positioned over the kidney area and upper thoracic region, respectively. The blood radioactivity levels were also measured by assaying serially collected blood samples in a deep-well scintillation counter.

The sequence of events and reciprocal relationships in normal animals are shown in Fig. 3*a*. Even with the data plotted on semilogarithm paper, the curves are not reduced to straight lines, showing that these functions are not of simple exponential character.

The same techniques were employed on rabbits which had previously been administered mercuric chloride at two different dosage levels. Fig. 3*b* (from an animal receiving 1.0 mg. per kilogram) shows a slower rate of accumulation of radioactivity in the kidney area and a slower rate of clearance. The excretion rate of radioactive Diodrast from the cannulated ureter is depressed and associated with relative oliguria. The externally recorded and serially sampled curves of blood radioactivity also showed decreased clearance rates and higher levels of retention. Curves in Fig. 3*c*, typical of animals receiving the higher dosage (2 mg. per kilogram) of mercuric chloride, indicate no active renal uptake and only a minimal decrease in blood radioactivity levels associated with complete anuria. The renal tubules, as might be anticipated, were almost universally obstructed by hematin and granular proteinaceous debris of desquamated tubular epithelial cells, on histologic examination.

In order to assess the effect of acute obstruction alone on blood clearance, similar tests were performed on rabbits with bilateral ureteral ligation, but without mercuric chloride administration. Fig. 3*d*, a typical tracing from one such animal, shows complete retention of activity in the kidney area, as would be anticipated, but the blood clearance curves of radioactivity closely parallel those in normal animals and are at only slightly higher levels. These findings indicate that in acute ureteral obstruction the kidneys are capable of clearing

almost all of the injected dose of radioactive Diodrast from the blood stream by concentrating and retaining this material wtihin the renal parenchyma, pelvis, and calyces.

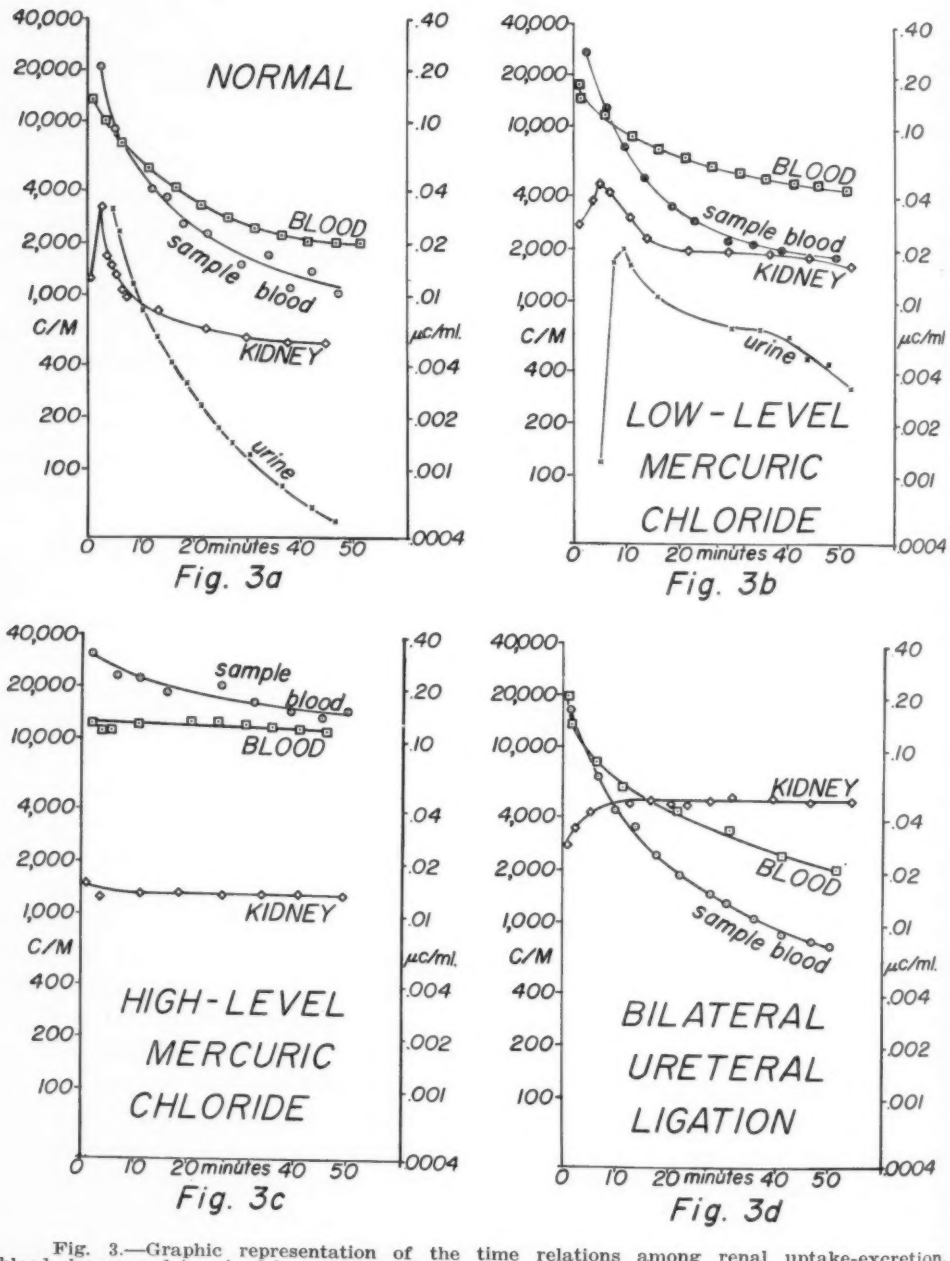


Fig. 3.—Graphic representation of the time relations among renal uptake-excretion, blood clearance determined by serial blood samples and by external measurements, and urinary excretion rates determined by radioassay of urine samples collected from cannulated ureters in normal animals (*a*), poisoned animals (*b* and *c*), and in animals with bilateral ureteral ligation (*d*).

Blood Radioactivity Measurements.—Two sets of determinations were made in the foregoing experiments (Figs. 3*a* and 3*d*) in order that blood clearance of radioactivity as determined by the external counting method might be compared with blood sampling techniques. In each case there was an apparent disparity between the curves attributable to several factors. The apparent differences during the first few minutes are related to the ratemeter capacitor lag, mechanical inertia of the recorder, and to the time required for thorough Diodrast mixing in the blood. After the first five minutes, the apparently higher levels of radioactivity measured externally as compared with well-crystal counts can be attributed to scatter radioactivity from the animal and to possible tissue retention of minute quantities of test substance. Regardless of these factors, the curves plotted from external measurements (c/m) reflect the actual changes in blood radioactivity levels (μc/ml.) with an accuracy quite satisfactory for clinical purposes. The external technique could be improved and made more exact by shielding the animal's body to minimize background radioactivity and by reducing lag factors in the measuring and recording equipment.

PRELIMINARY CLINICAL STUDIES

Equipment.—Three sets of radiation detection and recording equipment were employed: one for each kidney and a third to measure the clearance rates of the I^{131}-labeled agents from the blood. These units were of similar design and comparable sensitivity to those used in the animal studies. A deep-well scintillation counter was used to measure the gamma-ray activity in blood and urine samples.

Early Results With Radioactive Urokon and Diodrast.—In the initial clinical trials using radioactive Urokon, the renal uptake-excretion curves in normal subjects reflected only the vascularity of the organs and the rates of disappearance of the test material from the blood stream. Furthermore, when one patient with a known unilateral, nonfunctioning kidney was tested, there was little difference between the two kidney curves. Several other patients with various types of unilateral renal diseases were studied, and no significant differences were found unless the observation period was extended from 30 minutes to several hours. Only 10 to 15 per cent of the injected dose of Urokon I^{131} could be recovered in the patient's urine after 30 minutes, whereas in rabbits, over 90 per cent is recovered in 30-minute urine samples. With this demonstration that the rate of Urokon excretion in man is many times slower than in rabbits, clinical trials with Urokon were temporarily discontinued and similar studies were instituted using radioactive Diodrast. When the first normal patient was tested with Diodrast, it was obvious that the uptake-secretion curve was nearly identical to those found in normal rabbits. This discrepancy was explained by demonstrating that trace doses of radioactive Diodrast were excreted by normal individuals at rates many times faster than the same subjects could excrete comparable trace doses of radioactive Uroken. These findings obtained externally were confirmed by measuring the per cent excretion of the injected material in fractional urine samples. In addition, the rates of blood clearance

of both radioactive test agents, as measured externally, showed fairly close correspondence to values obtained simultaneously from radioassay of serial blood samples. Thus, for the practical purpose of providing a rapid test procedure useful in diagnostic urology, Diodrast was employed almost exclusively throughout this part of the investigation. However, for application in general medicine and for research, I^{131} Urokon, in doses used in urography, could be used to estimate glomerular function and Diodrast to indicate renal tubular secretory function.[2]

Important Factors in the Performance of the Diodrast Renogram on Patients.—1. In order that reproducible renograms might be obtained, it is essential to determine the precise location of the kidneys by means of an upright x-ray film of the abdominal region.

2. In order that comparative estimates of individual kidney function might be made, it is necessary to calibrate the instruments to respond equally to a standard radiation source of gamma rays and to position the counters in a manner which would permit measurements of equal volumes of tissue.

3. In early tests it was noted that there was a slight difference between the excretion segment of the right and left kidney curves. Diodrast is known to be absorbed and excreted partly by the liver, although it is mainly (85 to 90 per cent) recoverable in the urine. The influence of the liver radioactivity on the right renogram was demonstrated using Rose Bengal I^{131} (Fig. 4*c*), which shows both the blood clearance tracing and bilateral Rose Bengal renograms. The right renogram reflects and parallels to a lesser degree the hepatic curve due to recording radioactivity from the adjacent liver, while the left renogram is unaffected since Rose Bengal is not excreted by the kidneys. From a series of experiments performed to determine the optimal conditions of geometry to obtain right renograms which are least affected by the liver, it was determined that the posterior, upright position with the counters angled caudally toward both kidneys (as shown in Fig. 1*b*) was most suitable.

Standard Technique for Performing the Diodrast Renogram.—The patient is seated, leaning forward upon a comfortable support. Two scintillation probes are used, each directed caudally toward a kidney after x-ray localization (Fig. 1*b*). With the equipment used, the optimal dosage of radioactive Diodrast was 1 μc per 3 kilograms of body weight. The test dose is administered intravenously at a rate of 0.1 ml. per second. The radioactive material is diluted in normal saline, to provide a solution containing 20 μc per milliliter. Since the average dose is between 20 to 30 μc, the volume of solution required is 1.0 to 1.5 ml. With the decay factor considered, it is practical to prepare a volume of diluted material sufficient for a two-week period.

For the benefit of others who may wish to use this test in the future, it should be emphasized that it is not only necessary to adhere to the techniques described but also to realize that minor modifications are likely to be needed due to variations in sensitivity of slightly different types of instrumentation. Furthermore, it is essential that the test be performed *first* on a large number of normal subjects, in order to establish a baseline from which one may interpret

the significance of deviations from the patterns which occur in renograms from patients having various functional and/or structural abnormalities of the kidneys and ureters.

METHOD OF THE CLINICAL STUDY

The clinical studies consisted of the performance and interpretation of 125 renograms obtained from normal sujects as well as from patients with various renal disorders (absent kidney, non- or poorly functioning kidney, various degrees of hydronephrosis and chronic nephritides). Only a few of the more common conditions are discussed below. The Diodrast Renograms were correlated with standard renal function tests, such as creatinine or para-aminohippuric acid clearance, phenolsulfonphthalein excretion, and/or intravenous urography. Other laboratory tests included blood counts, urinalyses, and blood urea nitrogen and blood creatinine determinations. In many subjects the blood clearance of the radioactive agent was determined by radioassay of samples obtained after 3, 10, 20, and 30 minutes, as well as by the external use of a third scintillation counter directed toward the head or upper chest. The latter measurements aided in estimating over-all renal function and in evaluating the feasibility of the external method of determining blood clearance.

RESULTS AND INTERPRETATIONS

1. *Normal Diodrast Renogram.*—A simultaneously recorded typical Diodrast Renogram for a normal left kidney and the blood clearance curve are shown in Fig. 4*a*. Inspection of the renogram reveals marked similarity to the tracing obtained from normal rabbits, and the same interpretation of segments *A*, *B*, and *C* applies.

Although these three segments are readily distinguishable in nearly all normal individuals, there are minor variations from this typical renogram. In younger individuals the magnitudes of segments *A* and *B* are usually greater than in elderly patients whose renal vascularity and tubular function may be decreased. In instances where simultaneous recording of liver radioactivity is unavoidable, segment *C* in the right renogram may be less steep initially and may reach a plateau at a higher level than in the left renogram. This occurs most often in short individuals or when hepatomegaly is present.

2. *Nonfunctioning or Absent Kidney.*—A Diodrast Renogram typical of these abnormalities is shown in Fig. 4*b*. Since in these conditions few, if any, tubular cells are available for Diodrast uptake, the curve shows only a low initial vascular segment (*A*), an absent secretory segment (*B*), and a slowly falling excretory segment (*C*). It is interesting to note that the same renogram pattern is obtained when a comparable dose of Rose Bengal is administered to a patient with normal kidney function (Fig. 4*c*).

3. *Hydronephrosis.*—A characteristic pattern of the Diodrast Renogram is found in patients with hydronephrosis (Fig. 4*d*), provided that some renal tubular function remains.[3] The initial vascular segment (*A*) is usually definitely low, probably caused by a reduction in the amount of renal paren-

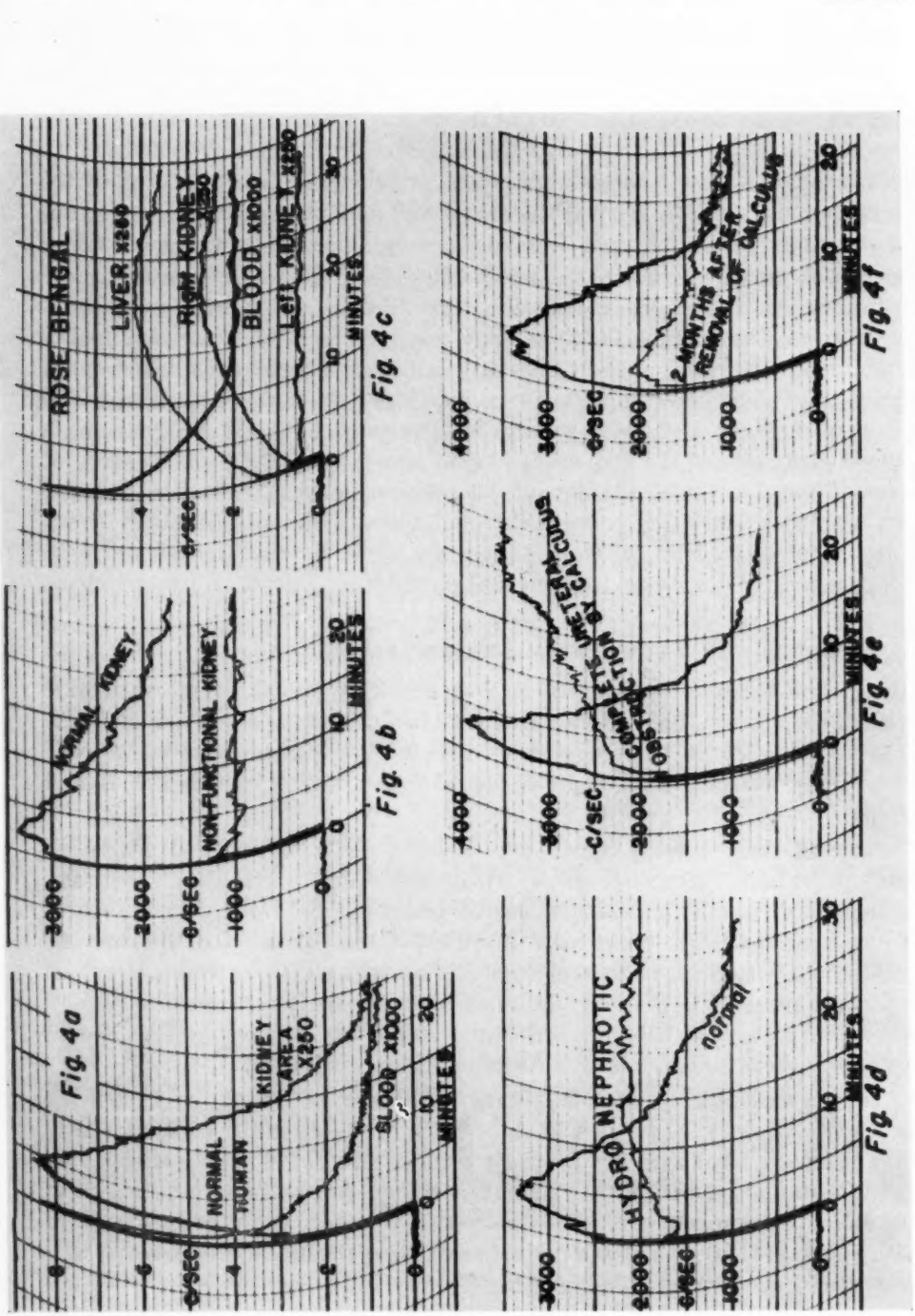


Fig. 4.—Typical examples of Diodrast Renograms in normal human subjects (*a*) and in patients with various renal-ureteral abnormalities (*b, d, e,* and *f*). Fig. 4*c* shows curves for blood clearance and liver uptake of I^{131}-tagged Rose Bengal together with bilateral Rose Bengal Renograms, the latter demonstrating the influence of the liver on the right renogram.

chyma and by an associated impairment of renal vascularity. The secretory segment (*B*) is prolonged due to impairment of tubular cell function and delayed outflow of urine from the pelvis. The downward slope of segment *C*, which is usually present, appears to indicate the degree of ureteral patency.

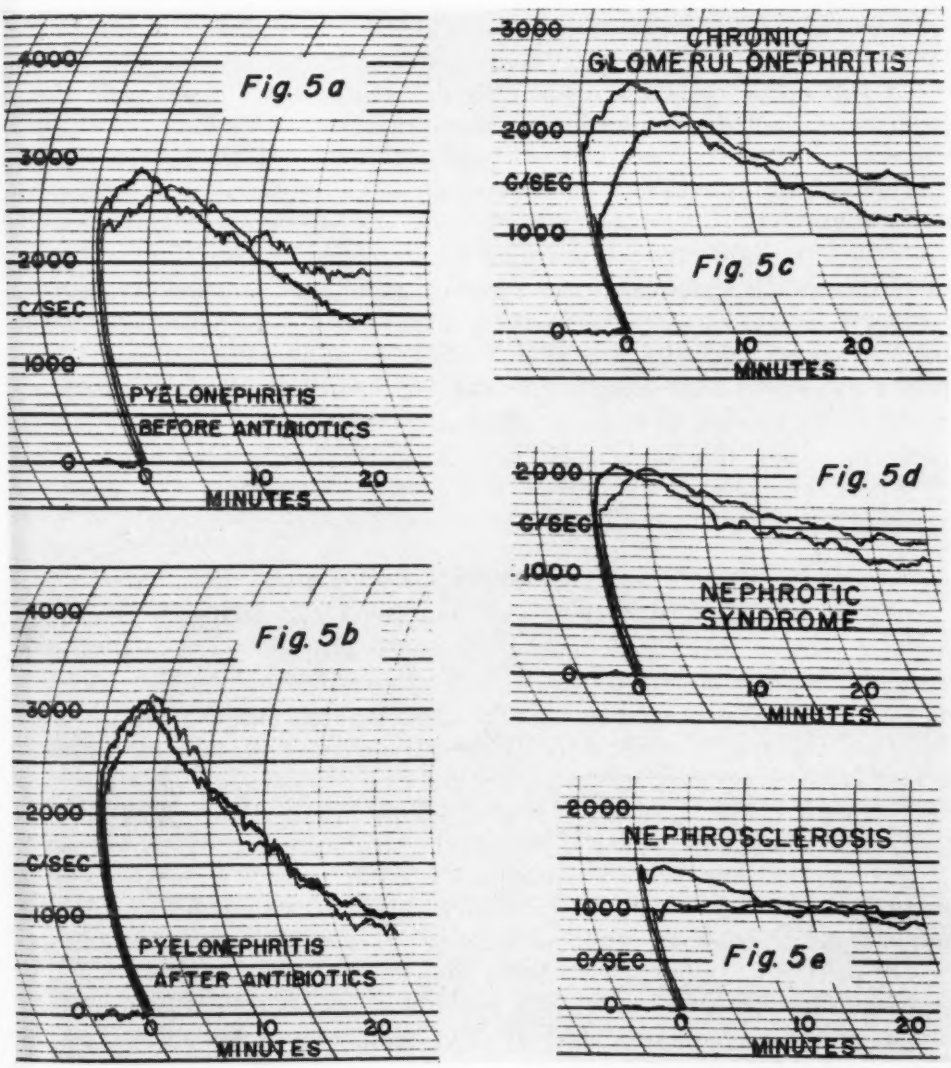


Fig. 5.—Renograms from an individual with acute pyelonephritis before and after antibiotic treatment and from three separate patients with other types of renal disease.

Fig. 4.—Typical examples of Diodrast Renograms in normal for blood clearance and liver uptake of I¹³¹-tagged Rose Bengal together with bilateral Rose Bengal Renograms, the latter demonstrating the influence of the liver on the right renogram.
ureteral abnormalities (*b*, *d*, *e*, and *f*). Fig. 4*c* shows curves

4. *Acute Ureteral Obstruction: (Calculus).*—An entirely different Diodrast Renogram is obtained in patients with this disorder (Fig. 4*e*). The initial vascular segment (*A*) is usually somewhat low, probably due to associated circulatory depression. Segment *B* of the curve continues to rise as the radioactive material accumulates in the renal tubules and pelvis and finally reaches a

plateau after 20 to 30 minutes or longer. Segment *C* is absent. A repeat renogram two months after surgical removal of the calculus shows return toward normal (Fig. 4*f*).

5. *Chronic Glomerulonephritis.*—The pattern of the Diodrast Renogram (Fig. 5*a*) shows decreased amplitude of the initial vascular segment. The tubular secretion segments (*B*) were prolonged and the slopes in segments *C* were decreased.

6. *Acute Pyelonephritis.*—Several individuals with unilateral acute pyelonephritis showed abnormal Diodrast Renograms, of which Fig. 5*b* is typical. There is a depression of segment *B* for the left kidney, and the slope of segment *C* in this curve is diminished. Following antibiotic therapy, the pattern of the entire curve returned toward normal (Fig. 5*c*), indicating improvements in renal tubular cell function and excretory capacity had occurred.

7. *Nephrotic Syndrome.*—The Diodrast Renogram presented in Fig. 5*d* shows a decreased amplitude in segment *A*, prolonged segments *B*, and shallow slopes in segment *C* which parallel the rate of blood clearance. These findings suggest diminished renal blood supply and impairment of tubular cell secretion.

8. *Nephrosclerotic Syndrome.*—Several elderly patients having normal intravenous pyelograms were tested. Diodrast Renograms in these patients (Fig. 5*e*) consistently showed diminished, initial vascular segments (*A*), absent or decreased segments (*B*), and abnormally slow excretion segments (*C*).

DISCUSSION

The use of radioactive (I^{131}-tagged) Diodrast for estimation of renal function and patency of the upper urinary passages, with use of external gamma-ray scintillation counting techniques, was an almost self-apparent analogy to the radioactive Rose-Bengal test of liver function and biliary tract patency.[1]

When the liver is affected by diffuse parenchymal disease, the degree of damage is relatively uniform throughout the entire organ; hence, a liver function test determines not only the over-all ability of the organ with respect to that test, but probably that of any representative portion of the liver also.

The two kidneys, being anatomically separate, may be affected quite individually by various diseases or pathologic states (infection, urinary obstruction, vascular occlusion). Standard renal function tests measure certain functions of both kidneys but fail to detect individual abnormalities.

Needle biopsy of the liver, with experience, has become a relatively innocuous procedure, and may yield much valuable information concerning the over-all state of this rather homogeneous organ.[4] Needle biopsy of the kidney is not attempted nearly so often and, at present, is usually deemed considerably more dangerous.

The function of each kidney individually is usually estimated by intravenous pyelography, but the results are quite often subject to misinterpretation. Retrograde pyelography, by cystoscopy and ureteral cannulation, portends many of the hazards of a major surgical procedure and is rarely considered advisable in a seriously ill patient. The quantities of contrast media required for successful roentgen urography also engender an additional

risk of some magnitude.[5] Yet, in clinical situations requiring nephrectomy (such as instances of malignant renal tumor, Goldblatt kidney producing hypertension or pyonephritis) it is imperative to be certain that the remaining kidney is capable of sufficient function to maintain life. A nontraumatic clinical test for measuring differential renal functions would therefore hold great promise for use in urological practice.

Following the development of such a test with radioactive Diodrast in rabbits, the modifications of techniques necessary for clinical application presented different problems from those encountered during clinical trials with the radioactive Rose-Bengal liver function test. Factors of geometry were far more critical in man than in rabbits, and in order to obtain reproducible clinical results, it was necessary to localize the kidneys accurately (x-ray) and to direct the right kidney detector head downward to avoid the simultaneous recording of Diodrast uptake in adjacent liver tissue. Fortunately, the rates of uptake and excretion from liver tissue were slower than those for the kidneys, and only 10 to 15 per cent of the test dose was excreted by way of the liver. Thus, the right and left renograms were only slightly different. Furthermore, the rates of Diodrast liver uptake and excretion did not appear to be altered significantly by severe liver diseases such as advanced cirrhosis. However, further investigation is needed to verify this statement.

These trials did show, however, that radioactive Rose Bengal, though not excreted by the kidneys, may be used as a preliminary testing agent for estimating initial blood dilution levels of renal area vascularity. From this baseline, then, the active renal uptake of an equal microcurie dose of radioactive Diodrast may be estimated with greater accuracy. (This technique is identical in theory with the use of radioiodinated serum albumin [RISA] in establishing a blood dilution baseline for radioactive Rose-Bengal liver uptake-excretion studies.[6])

During the clinical part of this study, emphasis was placed on developing the Diodrast I^{131} Renogram as a rapid (15 to 30 minutes) test procedure for use primarily in the field of urology, which required only *qualitatively* reproducible results. Further animal studies are now in progress with similar radioisotope techniques, attempting to quantitate rates of renal blood flow, glomerular filtration, and tubular excretion and reabsorption.

A clinical evaluation of the Diodrast Renogram in over 200 additional urological patients conducted by one of the authors (C. C. W.) is soon to be published separately.[7] In paraplegic patients having urinary tract disorders of neurogenic origin, preliminary studies have shown that the Diodrast Renogram may indicate upper urinary tract obstruction according to our original interpretation. In such cases the accumulation of radioactivity in the renal areas or the slowness of its removal is frequently due to ureteral reflux and stasis in the kidney pelvis, rather than by actual upper urinary tract obstruction.

Furthermore, analysis of renograms from patients with various forms of nephritis shows that the steepness of the excretory lag (segment *C*) depends primarily on adequate renal tubular cell function and is modified secondarily by obstructive processes which decrease the rates at which the test substance

leaves the kidney areas. Therefore, in the interpretation of Diodrast Renograms, one must be especially careful in respect to the excretory segment (*C*) and its relation to the secretory segment (*B*). Unless there is a distinct secretory lag in the renogram indicating active tubular cell function, a slowly falling excretory leg does not necessarily indicate partial obstruction in the upper urinary passages. However, if there is no neurogenic uropathy and the patient has good tubular function, as indicated by segment *B* in the renogram, a diagnosis of upper urinary tract obstruction or stasis may be made. Conversely, a renogram with all three segments in the normal range strongly indicates the absence of upper urinary tract obstruction or stasis and also confirms the presence of normal renal vascularity and tubular cell function.

Several additional clinical applications of these external gamma-ray counting techniques in urology were given preliminary trials during this study. These included estimation of residual urine, demonstration of the phenomena of ureteral reflux, measurement of urinary excretion of Diodrast and/or Urokon in voided or catheterized urine specimens, as well as estimation of the blood clearance rates determined by external counting as compared with radioassay of serially collected blood samples. Each of these possible practical applications appears to have potential merit. However, more work is needed to evaluate their accuracy and clinical usefulness.

Another promising application of the Diodrast Renogram is its use as a screening procedure in patients with hypertension. Since most renal causes of hypertension are associated either with abnormalities of renal function or lesions causing obstruction in the upper urinary passages, this test should be most useful in finding the 5 to 10 per cent of young hypertensives who have unsuspected urological disorders. Only these individuals might then warrant further investigation, whereas the great majority might be spared the otherwise necessary complete urological study including intravenous and retrograde pyelography.

Probably the most significant aspect of this investigation is that it demonstrates once again the fundamental usefulness and clinical applicability of external gamma-ray scintillation counting and recording techniques. These procedures permit the continuous measurement of multiple physiologic events occurring simultaneously in various organ systems. The techniques now adapted for the qualitative estimation of kidney and liver functions appear feasible for determining dynamic, biologic functions in other organs and organ systems such as the lungs, thyroid, and reticuloendothelial system. These methods also might be applied to register organ blood volume or local tissue vascularity in a particular area and could possibly be adapted to measure many other important physiologic phenomena, wherein knowledge of rates of blood clearance and tissue accumulation or disappearance of metabolic or diagnostic test substances would provide a better understanding of the physiologic functions involved.

SUMMARY

The radioactive Diodrast Renogram test described in this report is an application of external gamma-ray scintillation counting techniques to estimate the

vascularity, tubular cell function, and patency of the upper urinary passages in each kidney area separately. The principles, methods, and equipment are basically the same as those used in the radioactive Rose-Bengal liver uptake-excretion test. Experiments in rabbits have revealed that tracer doses of either Diodrast or Urokon, labeled with I^{131}, can be employed as test substances. Tracings of renal uptake and excretion in certain specific renal abnormalities have characteristic patterns which reveal the extent and nature of the existing functional impairment. Animal studies also demonstrate that blood clearance rates of I^{131}-labeled Diodrast may be detected externally, thus providing a rapid estimate of bilateral renal function. The combined procedure of determining renal uptake and excretion of Diodrast in each kidney plus simultaneous external measurement of blood clearance provides a new tool for further investigations in renal physiology on a dynamic basis.

Following successful preliminary trials in rabbits, the methods were adapted for clinical use into a safe, rapid (15 to 30 minutes) procedure which may be performed in the office, clinic, or hospital with little or no discomfort to the patient. The test provides qualitatively reproducible data on the vascularity, tubular cell secretory function, and patency of the upper urinary passages in each kidney area individually which otherwise must be determined by intravenous pyelography and a battery of kidney function tests or, in many instances, by cystoscopy, bilateral ureteral catheterization, and retrograde pyelography. In addition to yielding information of diagnostic value, the test may be repeated at intervals to follow the patient's clinical progress pre- and postoperatively and as influenced by therapeutic measures.

We are grateful to Dr. Franz Bauer, Chief of Radioisotope Service, Veterans Administration Center, Los Angeles, and to Dr. Willard E. Goodwin, Chief of the Urological Service and Professor of Urology, UCLA School of Medicine, for their cooperation, and to Mrs. Marianne Lederer of Dr. Bauer's group for technical assistance. Mr. Clifton W. Reed and Mr. Lawrence R. Curtis deserve credit for the loan of an R-C scintillation detector and ratemeter, used in clinical studies. We wish to express our gratitude to Drs. Benedict Cassen and Norman S. MacDonald of the Atomic Energy Project and School of Medicine, University of California at Los Angeles, for repeated consultation and valuable advice. Finally, we are grateful to Dr. Donalee L. Tabern and Mr. Butler Sanchez of Abbott Laboratories for generously supplying the radioactive Rose Bengal, Diodrast, and Urokon used in these studies.

REFERENCES

1. Taplin, G. V., Meredith, O. M., Jr., and Kade, H.: The Radioactive (I^{131}-tagged) Rose Bengal Uptake-Excretion Test for Liver Function Using External Gamma-Ray Scintillation Counting Techniques, J. Lab. & Clin. Med. **45**: 665-677, 1955.
2. Porporis, A. A., Elliott, G. V., Fischer, G. L., and Mueller, C. B.: The Mechanism of Urokon Excretion, Am. J. Roentgenol. **72**: 995-1003, 1954.
3. Smith, Homer: The Kidney: Structure and Function in Health and Disease, London, 1951, Oxford University Press.
4. Morey, D. A. J., Means, R. L., and Plummer, K.: Effectiveness and Complications of Needle Biopsy of the Liver, J. A. M. A. **158**: 1489-1491, 1955.
5. Pendergrass, E. P., Hodes, P. J., Tondreau, R. L., Powell, C. C., and Burdick, E. D.: Further Considerations of Deaths and Unfavorable Sequelae Following the Administration of Contrast Media in Urography in the United States, Am. J. Roentgenol. **74**: 262-287, 1955.
6. Taplin, G. V., Meredith, O. M., Jr., Kade, H., Westover, J. L., Hansen, R. A., and Bennett, L. R.: The Radioactive (I^{131}-labeled) Rose Bengal Liver Uptake-Excretion Test. Its Use in the Evaluation of Hepatic Function, Peaceful Uses of Atomic Energy, August, 1955, Proceedings of the International Conference in Geneva **10**: 355-358.
7. Winter, C. C.: A Clinical Study of a New Renal Function Test, The Radioactive Diodrast Renogram, J. Urol. **76**: 182-196, 1956.

PHOSPHOLIPID TURNOVER IN MAN AS AFFECTED BY DIFFERENCES IN THYROID FUNCTION

WARNER H. FLORSHEIM, PH.D., HERBERT F. SHULL, AND
M. E. MORTON, PH.D., M.D.
LOS ANGELES, CALIF.

IN RECENT publications Moser[1, 2] has applied the mathematical method of Zilversmit[3] to the calculation of plasma phospholipid turnover times in six normal and nine hyperlipemic patients. He concluded that in nephrotic patients turnover times were greatly prolonged, and that this was also true in a patient presenting the classical symptoms of myxedema. In the latter case the turnover time was reduced to normal by thyroid medication. Because of our interest in the relationship between thyroid function, lipid metabolism and atherogenesis[4] we have studied phospholipid turnover in a much larger series of patients whose thyroid function had been assessed by clinical and laboratory evaluation. We have also reexamined the assumptions made by Moser in his calculations and have attempted to validate his conclusions by an alternate independent calculation of turnover time in some of our patients.

Zilversmit's calculations require a knowledge of the changes in specific activity with time of both the immediate precursor and the product in a homogeneous system. These requirements are not readily satisfied in a study of serum phospholipid turnover in man. Animal experiments by Fishler[5] show that serum lecithin and sphingomyelin are formed and degraded in the liver, and the work of Popjak[6] and of Tolbert[7] indicates that the glycerophosphate fraction of the liver satisfies the requirements for the immediate precursor of liver lecithin as defined by Zilversmit.[3] However, the inorganic phosphate fraction satisfies the precursor requirements almost as well as does glycerophosphate, and the chief argument in favor of the latter is that it is formed during the in vivo breakdown of labeled lecithin.[6, 8] Very little is known of the mechanisms which maintain the disparity between the serum and liver lecithin : sphingomyelin ratios, but in tracer experiments it has been shown that the specific activities of liver and serum lecithin tend toward equilibrium within a few hours, while sphingomyelin is released more slowly.[9]

It has not been possible to date to calculate plasma phospholipid turnover from known turnover rates in the liver of animals. Moser has calculated plasma phospholipid turnover directly, however, by making the assumptions that urinary phosphate is representative of the precursor pool and that plasma

From the Radioisotope Service, Veterans Administration Hospital, Long Beach 4, Calif.; and the Department of Physiological Chemistry, University of California Medical Center, Los Angeles 24, California.

Supported in part by a grant (H-2013) from the National Heart Institute, U. S. Public Health Service.

Received for publication June 1, 1956.

phospholipids equilibrate rapidly with those of the liver. He observed a low turnover in a single myxedemic, and the turnover in this patient was increased by thyroid therapy. This agrees with older work by Flock, Bollman, and Berkson,[10] who found a direct relationship between liver phospholipid turnover and thyroid function in the rat. While the work of Barker[11] indicates that pancreatic as well as thyroid function influences the turnover of phospholipid, we decided to investigate further the relationship between thyroid function and the apparent plasma phospholipid turnover, calculated by Zilversmit's method as used by Moser. We also compared the turnover times computed by this method with those calculated from the clearance of intravenously injected labeled phospholipids.

MATERIALS AND METHODS

Turnover studies were carried out on patients whose thyroid function was clinically established. The 24-hour I^{131} uptake by the thyroid, the per cent of serum radioactivity represented by thyroxine,[12] and the serum cholesterol level were measured in each case to support the diagnosis.

The apparent plasma phospholipid turnover time was calculated in 62 patients. Each patient received 100 to 160 μc of P^{32} labeled phosphate* intravenously. Blood specimens were obtained at intervals from 1 to 72 hours and the serum fractionated into lipid and TCA-soluble phosphorus by the method of Zilversmit and Davis.[13]

Since earlier experiments[14] had shown the specific activity versus time curves of serum lecithin and sphingomyelin to have very similar shapes, no attempt was made in this study to resolve the phospholipid fraction into its components. We also found that the amount of organically bound phosphorus in the TCA-soluble fraction was negligible.

In most patients the maximum specific activity of lipid P^{32} was reached after about 48 hours, and lipid and phosphate specific activities were essentially equal at that time. Turnover times were calculated by Zilversmit's method for the 6- to 48-hour interval with use of the total acid soluble phosphorus as the precursor.

The half-life of injected labeled phospholipids was also determined directly. Phosphorus-labeled rat liver phospholipids purified by repeated acetone precipitation were emulsified in the patient's own serum and reinjected. Nine such experiments were carried out in 5 volunteers. Several other phospholipid preparations were used in rabbits. The serum lipid phosphorus specific activity was followed for 72 hours after injection. The curves so obtained were resolved into two linear components, on a semilogarithmic scale, one dominant for 6 hours after injection (assumed to represent distribution to various tissues) and a slower phase presumably representing metabolic disposal.

RESULTS AND DISCUSSION

The turnover times calculated by the Zilversmit technique are presented in Fig. 1. In the euthyroid group of 43 patients the apparent phospholipid turnover time was 74 ± 24 hours.† The ten hypothyroid patients had turnover times averaging 80 ± 33 hours, which does not differ significantly from the euthyroid group. The nine hyperthyroid patients investigated had a significantly shorter turnover time, averaging 47 ± 17 hours ($t > 5$).[15] This agrees with the findings of Flock and co-workers[10] in the rat.

*Furnished by the Oak Ridge National Laboratory of the U.S. Atomic Energy Commission.
†Mean ± standard deviation.

Since the plasma phospholipid levels were lower in the hyperthyroid patients than in the other two groups, the milligrams of lipid phosphorus turned over per liter of serum in one hour was calculated. The resultant values were:

euthyroids:	1.67 ± 0.72 mg. per liter per hour
hypothyroids:	1.52 ± 0.64 mg. per liter per hour
hyperthyroids:	1.94 ± 0.49 mg. per liter per hour

The differences between the three groups were not statistically significant. Moser's conclusion that the absolute quantity of plasma phospholipid renewed per hour is relatively independent of the patient's state of thyroid function is thus confirmed.

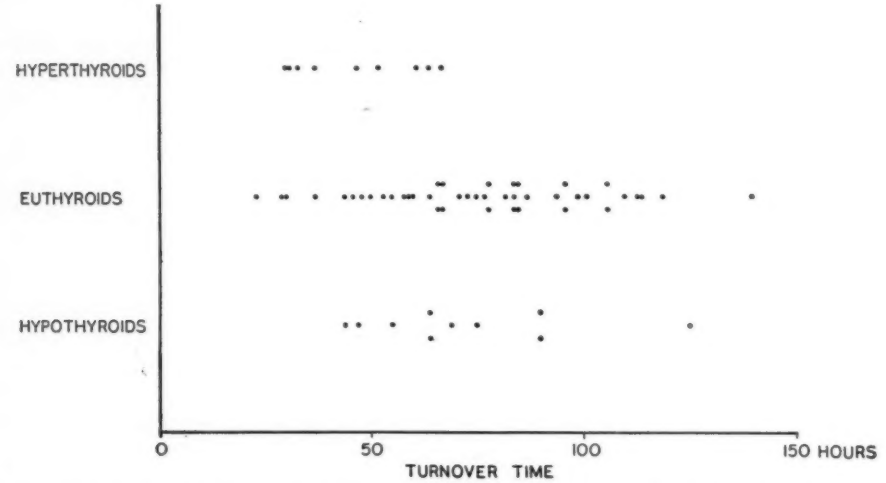


Fig. 1.—Distribution of observed phospholipid turnover times in 9 hyperthyroid, 43 euthyroid, and 10 hypothyroid individuals.

In order to check on the validity of the assumptions inherent in applying Zilversmit's method to the calculation of serum phospholipid turnover, we decided to compare the results obtained above with the turnover values calculated by measuring the disappearance of injected phosphorus-labeled phospholipids from the serum. Zilversmit[16] has demonstrated that the half-life of injected egg yolk phospholipid in dog serum is the same as that of transfused labeled dog serum lipoprotein. Zilversmit's investigation covered only the first six hours after injection, i.e., what may be assumed to be the distribution phase. In the present study a number of experiments in rabbits were done, and the half-life of injected phospholipid was observed to average 2.0 ± 0.4 hours for the rapid phase, regardless of whether labeled rabbit serum lipoproteins, or rat serum-, rat liver-, or mouse liver-phospholipids were used. The half-life calculated for the slower (disposal) part of the disappearance curve was 36 ± 8 hours, independent of the source and composition of the labeled phospholipids. The fact that foreign phospholipids follow the same metabolic pattern as the host's own serum phospholipids indicates a rapid exchange of

the injected phospholipids with the phospholipids of the serum lipoproteins and of the liver.[14] Weinman[17] has shown that the choline and phosphate moieties of the phospholipid molecule are turned over at the same rate. For this reason, and in view of the large inorganic phosphate pool, it is clear that the equilibration of injected phospholipid with endogenous material cannot be due to breakdown to phosphate followed by reincorporation of the label. The equilibrium which is established must be due to the exchange of relatively large units.

Since the experiments on rabbits indicated no metabolic differentiation between injected and endogenous serum phospholipids, a serum phospholipid turnover rate could be calculated from the experiments on the five patients who received labeled rat liver phospholipids equilibrated with the patients' own serum. In 6 assays on 3 euthyroid patients and in 3 assays on 2 hypothyroids we found a mean phospholipid turnover rate of 2.43 ± 0.85 mg. lipid phosphorus per liter of serum per hour. This value does not differ significantly from the values calculated by means of the Zilversmit method. The agreement between the two methods is even better in the case of the two volunteers on whom both tests were carried out:

PATIENT	THYROID STATUS	TURNOVER BY	
		ZILVERSMIT	CLEARANCE
A. Z.	Euthyroid	2.02 mg. per liter per hour	2.33 mg. per liter per hour
C. M.	Hypothyroid	2.77 mg. per liter per hour	2.50 mg. per liter per hour

The differences are well within the experimental errors involved in the determinations. It thus appears that a valid determination of the plasma phospholipid turnover in patients can be made by the method described by Moser. However, no clear relationship of diagnostic value between thyroid function and phospholipid metabolism can be deducted from our results.

SUMMARY

An apparent serum phospholipid turnover time was calculated by the Zilversmit technique in a series of patients with carefully documented thyroid function. The results show a significantly shorter turnover time in the hyperthyroid group compared to the hypo- and euthyroids. Since the hyperthyroids have proportionally lower serum phospholipid levels, the amount of phospholipid actually turned over per hour appears to be independent of thyroid function.

A comparison with the turnover times calculated from the clearance of peripherally injected labeled phospholipids indicates that the assumptions made in calculating turnover by Zilversmit's method are valid.

REFERENCES

1. Moser, H. W.: Phospholipide Turnover in Man, J. Clin. Invest. 31: 651, 1952.
2. Moser, H. W., and Emerson, K., Jr.: Estimation of the Phospholipid Phosphorus Turnover Time in Man, J. Clin. Invest. 34: 1286, 1955.

3. Zilversmit, D. B., Entenman, C., and Fishler, M. C.: On the Calculation of "Turnover Time" and "Turnover Rate" From Experiments Involving the Use of Labelling Agents, J. Gen. Physiol. **26:** 325, 1943.
4. Florsheim, W. H., Morton, M. E., and Goodman, J. R.: The Effect of Thyroid Ablation Upon Serum Cholesterol and Lipoprotein Spectrum, Am. J. M. Sc. In press.
5. Fishler, M. C., Entenman, C., Montgomery, M. L., and Chaikoff, I. L.: Formation of Phospholipid by Hepatectomized Dogs as Measured With Radioactive Phosphorus, J. Biol. Chem. **150:** 47, 1943.
6. Popjak, G., and Muir, H.: In Search of a Phospholipin Precursor, Biochem. J. **46:** 1, 1950.
7. Tolbert, M. E., and Okey, R.: The Relative Rates of Renewal of Choline and Phosphate in Liver Phospholipide in the Rat, J. Biol. Chem. **194:** 755, 1952.
8. Dawson, R. M. C.: Liver Glyceryl Phosphorylcholine Diesterase, Biochem. J. **62:** 689, 1956.
9. Zilversmit, D. B., Entenman, C., and Chaikoff, I. L.: The Measurement of Turnover of the Various Phospholipides in Liver and Plasma of the Dog, J. Biol. Chem. **176:** 193, 1948.
10. Flock, E. V., Bollman, T. L., and Berkson, J.: Effect of Thyroxine and Thiouracil on the Rate of Phospholipid Turnover in the Liver of the Rat, Am. J. Physiol. **155:** 402, 1948.
11. Barker, W. F., Rogers, K. E., and Moore, F. D.: Effect of Pancreatectomy on Phospholipid Synthesis in the Dog, Arch. Surg. **61:** 1151, 1950.
12. Morton, M. E.: Measurement of Thyroxin Synthesis With I^{131}, California Med. **78:** 277, 1953.
13. Zilversmit, D. B., and Davis, A. K.: Microdetermination of Plasma Phospholipides by Trichloroacetic Acid Precipitation, J. Lab. & Clin. Med. **35.** 155, 1950.
14. Florsheim, W. H., and Morton, M. E.: The Stability of Human Serum Lipoproteins as Measured With Radiophosphorus, Geneva, 1955, Abstr. Intern. Conf. on Peaceful Uses of Atomic Energy **10:** 496.
15. Fisher, R. A.: Statistical Methods for Research Workers, London, 1936, Oliver & Boyd.
16. Zilversmit, D. B.: The Fate of Intravenously Administered Egg Yolk Phospholipids in Dogs, Arch. Biochem. **46:** 261, 1953.
17. Weinman, E. O., Chaikoff, I. L., Entenman, C., and Dauben, W. G.: Turnover Rates of Phosphate and Fatty Acid Moieties of Plasma Phospholipides, J. Biol. Chem. **187:** 643, 1950.

LABORATORY METHODS

DIRECT DETERMINATION OF C^{14} AND S^{35} IN BLOOD

WILLIAM W. BURR, JR., PH.D., AND DONALD S. WIGGANS, PH.D.
DALLAS, TEX.

INTRODUCTION

IN BIOLOGIC experiments employing tracer techniques, it is frequently desirable to determine periodically the increase or decrease in activity of the blood following the administration of a radioactive substance. With a white rat as the experimental animal, small amounts of blood are easily obtained from the tail vessels. The size of the available sample makes a direct mounting procedure desirable. If two isotopes are employed, and each is to be determined on the same sample, it becomes even more desirable to use a direct procedure which does not necessitate the separation of the two labeled compounds. In order to measure C^{14} activity in blood, a method employing a porous support has been used in this laboratory.[1] Porous supports have been described by a number of investigators[3-5] for solutions of active materials. In this report a procedure as used for blood is described, the method is evaluated for reproducibility, and comparative values are given which were obtained by this direct method and by oxidation of the blood followed by the precipitation of the CO_2 as $BaCO_3$.

In connection with another study in which labels of C^{14} and S^{35} were being used in the same system, a simple procedure was needed to evaluate the proportion of the total activity due to each isotope. For this purpose the direct method has been adapted for determination of C^{14} and S^{35} in the same sample utilizing the different rates of decay.[6]

METHODS

Preparation of Mounts.—In the preliminary C^{14} studies heparinized rat blood was labeled in vitro by the addition of DL-alanine-1-C^{14}. The remainder of the C^{14} blood was obtained from animals which had received an oral administration of labeled palmitic acid in olive oil. When the blood fat was labeled, a 1:1 dilution of blood with normal saline containing 0.8 mg. of heparin per milliliter was routinely made. Aluminum planchets, 1 inch in diameter, were used for all direct plating. A porous support of lens paper was placed in the planchet, and the planchet and paper were weighed. Lens tissues for this purpose were cut in batches to the size of a quarter. This allowed for a slight margin between paper and planchet which improved reproducibility.

From the Department of Biochemistry, the University of Texas, Southwestern Medical School, Dallas, Tex.

Supported by Contract No. AT-(40-1)-1988 between the U. S. Atomic Energy Commission and the University of Texas, Southwestern Medical School.
Received for publication May 25, 1956.

Two drops of the diluted blood were applied near the center of the planchet and the lens paper was placed over the blood. The blood distributed throughout the paper and the mounts were allowed to dry in room air and were weighed. Drying at room temperature in the absence of a desiccant minimized the number of tissues which curled. Three or more planchets were prepared for each sample. When aliquots were oxidized to CO_2 for comparison with the direct technique, either a wet oxidation using a modified Van Slyke-Folch oxidizing mixture[7] or a dry combustion was employed. In both procedures the CO_2 was trapped in NaOH and precipitated as $BaCO_3$. The activity was determined on pressed mounts[2] of infinite thickness.

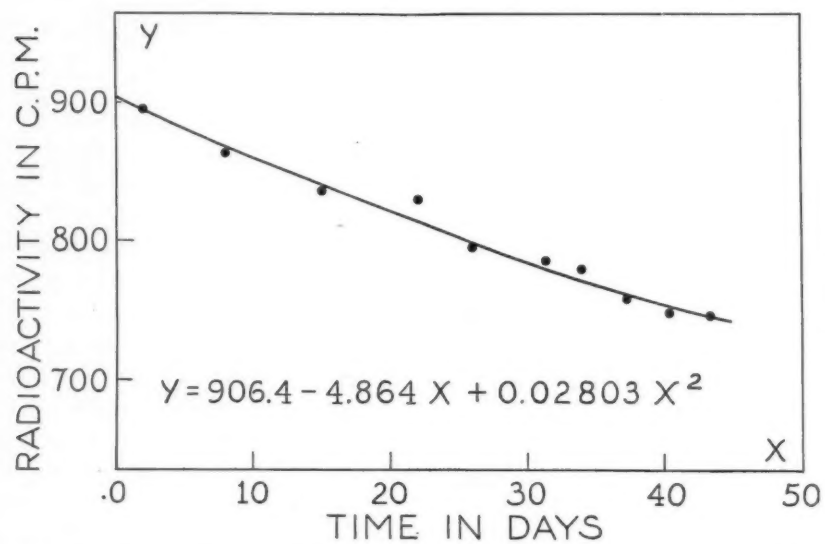


Fig. 1.—Decay curve of *sample 1A* of Table II. ●, radioactivity as measured. The curve is the calculated parabola of best fit indicated by the equation.

Determination of Radioactivity.—All samples were counted with either a windowless gas flow counter or a thin window (1.4 mg. per square centimeter) Geiger-Muller tube. Samples were routinely counted for a minimum of 10,000 counts. When the standard deviation of a total count divided by the counting time is used for the counting rate error of the sample (a) and the background (b), the combined error of the count corrected for background is $\sqrt{a^2 + b^2}$. In all cases when the counts per minute (CPM) were twice background or more, the error was not greater than 1 per cent of the counting rate. When $BaCO_3$ from oxidized samples was assayed, the results are expressed in specific activity which is defined here as the calculated CPM per milligram of material oxidized. The specific activity calculated was the (observed CPM corrected for background) × (milligrams $BaCO_3$ from oxidation) ÷ (milligrams of sample oxidized).

The activity of each planchet was corrected for self absorption on the basis of curves prepared from C^{14}-labeled blood. To make this correction a series of mounts of various weights was prepared and counted. The activities were plotted against the dry weights of the blood mounts and all values were corrected to the same thickness on the basis of this curve. This graphical method has been described elsewhere.[8]

Calculation of S^{35} and C^{14} Ratios.—Blood samples containing S^{35} and C^{14} were combined in vitro in a series of known proportions and plated for counting. Duplicate samples were periodically counted and the activities plotted against time. To minimize individual counting error, these points were fitted to a parabola by the standard methods of curve fitting. Calculations were made at the end of forty days. The total activities on the

initial and final days as predicted by the curves of best fit were used to determine the carbon and sulfur activities. Since two isotopes are involved, the total activity (y) represents the sum of the individual activities of the S^{35} (y_s) and C^{14} (y_c); or $y = y_s + y_c$. As the S^{35} decays, y_s becomes smaller (y_s'). The value of y_s' can be related to the original activity y_s by a constant (k), which is dependent on the time interval between assays and the rate of decay; $y_s' = ky_s$. Since k can be calculated,* any two points on the curve can be employed to solve simultaneous equations for y_s and y_c.

$$Y_1 = y_s + y_c$$
$$Y_2 = ky_s + y_c$$

A curve representative of the sample is shown in Fig. 1. The S^{35} and C^{14} activities were calculated as well as the ratio of the S^{35} activity to that of the C^{14}.

RESULTS AND DISCUSSION

Reproducibility.—When three planchets were prepared from a blood sample, the mean activity of the three was taken as the measure of the activity. In a series of 102 such mounts when the activity was at least twice background, the average deviation of a count from its mean was 2.27 per cent of the count. The standard deviation for these variations was ±2.0 per cent of the count. Of the 102 planchets, 98 showed deviations from their respective means of not greater than 6 per cent.

Variations were found to exist between different sheets of paper, and reproducibility of weight must be established or the weight of each paper determined. Five series, each of 12 papers, were found to range in weight from 5.55 to 6.60 mg. with standard deviations of 0.04 to 0.11 mg. In terms of thickness, these limits of deviation would be 0.008 to 0.021 mg. per square centimeter. With a dried blood sample of 6 mg. or more, these deviations would represent less than 1 per cent of the sample weight. For this reason, where the same sheet of tissue was used in comparative determinations, lens papers were not individually weighed but included in the planchet weights.

Comparison With Oxidative Methods.—The activities of two series of samples, which were assayed both by direct mounting and by methods employing oxidation to CO_2, are given in Table I. It can be seen from the ratios of the direct counts to those of $BaCO_3$ that the two methods give equivalent results. It should be noted that the direct mounts of the different series are not of the same thickness and hence the two groups are not directly comparable.

Mixtures of S^{35} and C^{14}.—In experiments using two labeled compounds, it is usually necessary to separate the compounds in order to assay the activity of each isotope. It is desirable with small samples to differentiate the activities without prior compound separation. Attempts toward this goal were made on the basis of the per cent of the count which could penetrate a thin aluminum filter. This is theoretically possible due to the different maximum energies of the emitted β particles of the isotopes. However, the normal variation encountered in planchet counting sufficiently masked the measured effect to make the method unsuccessful.

---

*$k = e^{-\frac{0.693x}{t}}$ where x = time, and t = half-life of S^{35}.

TABLE I. BLOOD RADIOACTIVITIES AS DETERMINED BY DIRECT MOUNTING AND BY OXIDATIVE METHODS

SAMPLE	DIRECT MOUNT* (I)	$BaCO_3$ MOUNT† (II)	RATIO (I)/(II)
A-1	1,498	11,200	0.13
A-2	788	6,100	0.13
A-3	611	4,750	0.13
P-1	22	83.5	0.26
P-2	106	432.7	0.25
P-3	174	695.2	0.25

*CPM less background. Series A planchets were prepared from alanine-1-C^{14} labeled bloods and plated at 0.97 mg. per square centimeter or corrected to this thickness. P planchets contained periodic blood samples from a rat which had received palmitic acid-1-C^{14}. These mounts had a thickness of 1.75 mg. per square centimeter or were corrected to this value.

†Specific activity in CPM.

A successive series of assays proved to be satisfactory using the direct porous support as has been described. Based on 10 sequential determinations of each planchet over a 40-day decay period, the activities of both isotopes were calculated and related to predicted values for known mixtures of blood containing S^{35} and C^{14} (Table II). In order to minimize the influence of counting error for any single determination, a curve of best fit was calculated for each series. Thus all measurements made during the period exert influence on the curve. The initial and final activities for the 40-day period were calculated from the equation of the curve and were employed to determine values attributable to each isotope. The parabolic function proved to be a satisfactory estimate for a relatively short portion of the curve, which is exponential. The mathematical manipulations involved are less complicated than for the exponential function, and results are sufficiently accurate as long as the curve is not extrapolated. The time interval over which the decay is to be determined is arbitrary, and greater accuracy would be expected with a longer decay period. In the present series of determinations, the 40-day interval was

TABLE II. S^{35} AND C^{14} ACTIVITIES* IN DOUBLY LABELED BLOOD AS CALCULATED FROM A 40-DAY DECAY INTERVAL

SAMPLE	TOTAL ACTIVITY† 0 DAYS	TOTAL ACTIVITY† 40 DAYS	LOSS OF ACTIVITY DUE TO DECAY FOR 40 DAYS	ACTIVITY† AT 0 DAYS ATTRIBUTABLE TO S^{35}‡ (I)	ACTIVITY† AT 0 DAYS ATTRIBUTABLE TO C^{14}§ (II)	(I)/(II)	MEAN OF I/II FOR DUPLICATE SAMPLES	PREDICTED RATIO I/II FROM KNOWN COMPOSITIONS OF MIXTURES
1A	906.4	756.8	149.6	550.0	356.4	1.543	1.585	1.616
1B	816.7	679.1	137.6	505.9	310.8	1.627		
2A	1052.6	903.0	149.6	550.0	502.6	1.094	1.099	1.065
2B	1083.4	928.8	154.6	568.4	515.0	1.104		
3A	1229.5	1099.5	130.0	477.9	751.6	0.636	0.582	0.585
3B	1338.7	1212.8	125.9	462.9	875.8	0.529		

*All activities corrected for background and expressed in CPM.

†Calculated values from the equation of the curve of best fit for the series of 10 periodic determinations. Not corrected for thickness.

‡Loss of activity in 40 days $1 - e^{-\frac{0.693 \cdot 40}{87.1}}$ (For calculation at 40 days. Half-life of S^{35} = 87.1 days.)

§Calculated total activities at 0 days – Calculated S^{35} activities at 0 days.

selected because further time increments did not sufficiently increase the accuracy to justify the inconvenience of delay in obtaining the results. On the other hand, shorter time sequences of assays gave wider fluctuations between the calculated ratios and those predicted from the known compositions of the mixtures. The period of decay necessary for satisfactory results for a given sample will be governed in part by the frequency of individual determinations, the counting error inherent in the instrument of assay, and the magnitude of and the ratio between the activities. It may be anticipated that if the decaying S^{35} is in increasing abundance, the time required to approach accurate values will be lessened.

In Fig. 1, the decay curve is shown for a representative sample. The points indicate the actual counts from which the curve is calculated. It is evident that counting errors are minimized by the curve-fitting process. For example, if only the second, third, and fourth values had been obtained over a 14-day interval, the rate of decay would have appeared to be much less and therefore the calculated S^{35}/C^{14} ratio would have been smaller.

SUMMARY

A direct mounting procedure suitable for radioactive assay of small blood samples has been described in detail and its reproducibility evaluated. Results obtained with this technique have been related to oxidative methods.

The procedure has been extended to include samples in which both S^{35} and C^{14} are present. The basis of the counting schedule employed and the calculations required for determining the percentage of the activity contributed by each of the isotopes are discussed.

REFERENCES

1. Burr, W. W., Jr., Dunkelberg, C., McPherson, J. C., and Tidwell, H. C.: Blood Levels of Absorbed Labeled Fat and Chylomicronemia, J. Biol. Chem. **210**: 531, 1954.
2. Burr, W. W., Jr., and Marcia, J. A.: Preparation of Pressed Samples for Counting Carbon-14-Labeled Compounds, Anal. Chem. **27**: 571, 1955.
3. Entenman, C., Lerner, S. R., Chaikoff, I. L., and Dauben, W. G.: Determination of Carbon 14 in Fatty Acids by Direct Mount Technic, Proc. Soc. Exper. Biol. & Med. **70**: 364, 1949.
4. Fager, E. W.: *In* Symposium on the Use of Isotopes in Biological Research, Chicago, 1947, Univ. Chicago.
5. Leslie, W. B.: Experimental Use of C^{14}, Atomic Energy Commission, MDDC 674.
6. Tait, J. F., and Williams, E. S.: Assay of Mixed Radioisotopes, Nucleonics **10**: 47, 1952.
7. a. Van Slyke, D. D., and Folch, J.: Manometric Carbon Determination, J. Biol. Chem. **136**: 509, 1940.
   b. Calvin, M., Heidelberger, C., Reid, J. C., Tolbert, B. M., and Yankwich, P. E.: Isotopic Carbon, New York, 1949, John Wiley and Sons.
8. Yankwich, P. E., Norris, T. H., and Huston, J. L.: Correcting for the Absorption of Weak Beta-Particles in Thick Samples, Ind. & Eng. Chem., Anal. Ed. **19**: 439, 1947.

SOME TRANSDUCERS ADAPTABLE FOR WATER PLETHYSMOGRAPHY

FRANCIS S. CALIVA, M.D., PAUL V. GABEL, B.S., WILLIAM MUELLER, AND RICHARD H. LYONS, M.D.
SYRACUSE, N. Y.

INTRODUCTION

FOR the last three years certain electronic transducers have been used in our laboratory in conjunction with venous occlusion plethysmography. We believe that these devices have significantly added to the usefulness and accuracy of the procedure. In addition, they have permitted simultaneous recordings of other physiologic phenomena. We are therefore reporting these instruments in some detail.

METHODS

I. For use with a D.C. amplifier and recording system, the instrument shown in Fig. 1, *a* was devised. The transducer is constructed from a stainless steel tube (*C*), 14 cm. in length and 2.5 cm. in diameter. The top of this tube is capped with a piece of Lucite (*A*), through which a stainless steel rod (*D*), 16 cm. long and 2 mm. in diameter, is placed. A 1/16 inch hole (*B*) is provided for the escape of trapped air and to allow for free movement of the water column inside the cylinder. This unit is placed in the glass water outlet which is a part of most plethysmographs.

A 50 ohm 50 watt wire wound resistor (*R* in Fig. 1, *a*; R_1 in Fig. 1, *b*) is placed across the output (*F*), and the fluctuations of the height of the water column are detected as a voltage drop across R_1 and recorded through the D.C. amplifier, such as that available with the Sanborn polyviso cardiette.

Fig. 1, *b* is a schematic representation of the system in which the stainless steel tube is represented by the variable resistor, R_2. The system acts as a variable voltage divider, and the voltage, V, at the output of the circuit is determined by the ratio of R_1 and R_2. Thus, $V = \frac{R_1}{R_1 + R_2} \times E$ where $E = 6$ volt storage battery, $R_1 = 50$ ohm, 50 watt resistor, $R_2 =$ resistance across the stainless steel cylinder and rod.

The response of the instrument is calibrated by the placing of known volumes of water in the plethysmograph. The linearity depends upon the uniform dimensions of the steel cylinder and the glass tube. It is essential that water be in contact with the steel rod before calibration is begun. There is some drift of the

From the Department of Medicine, State University of New York, Upstate Medical Center, Syracuse, N. Y.
Received for publication April 23, 1956.

base line due probably to hydrolysis of the water by the direct current, but this is negligible for most work. A sample calibration of 10 ml. volumes of water is shown in Fig. 2.

II. For recorders using a carrier-amplifier system, a wheatstone bridge type of transducer was utilized in conjunction with a Model III Grass electroencephalograph adapted to plethysmography according to the method of Simeone and associates.[1] This made it possible to study simultaneously the blood flow to a digit and to a larger part such as the foot or hand.

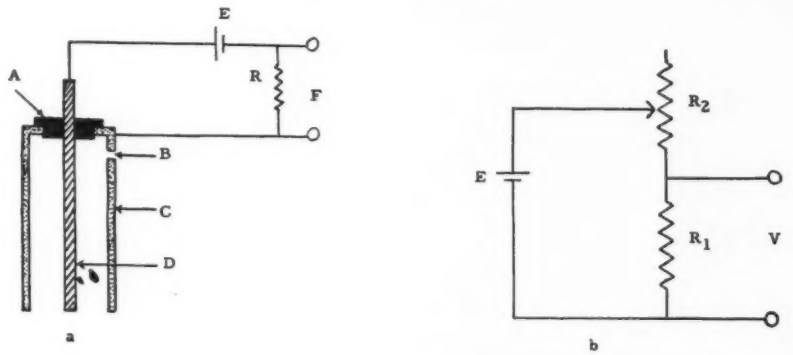


Fig. 1.—Schematic representation of transducer used with D.C. amplifiers.

The transducer (Fig. 3) consists of two aluminum rods (*C*), 0.6 cm. in diameter and 14 cm. in length. The rods are separated by two pieces of ¼ inch Lucite (*A* and *B*), which maintain a uniform distance of 6 mm. between them and which also function as insulators.

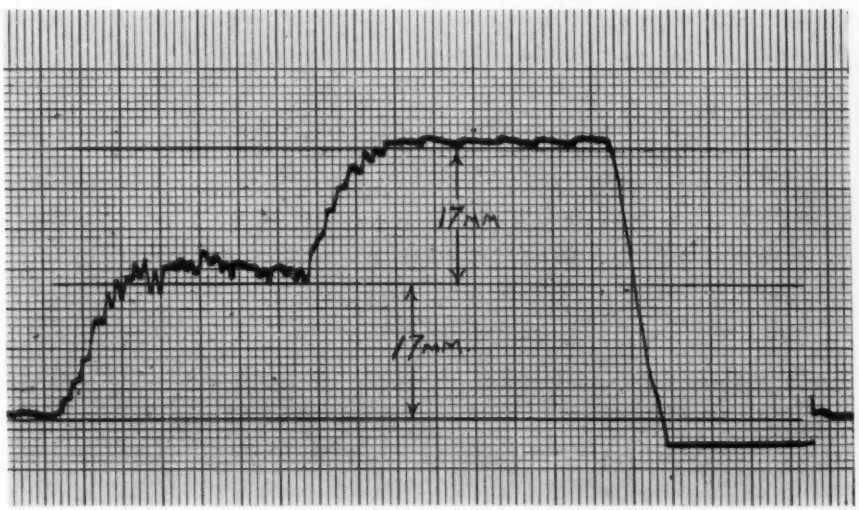


Fig. 2.—Actual calibration of instrument shown in Fig. 1, *a* and *b*.

The aluminum rods are placed in parallel with one leg (R_1) of a wheatstone bridge which is made up of four 120 ohm 1 per cent resistors (R_1, R_2, R_3,

R_4). In the use of an A.C. amplifier as a recorder, it is necessary to convert the bridge and recorder into a carrier frequency system if sustained phenomena are to be measured. This has been accomplished by the balance demodulator unit available with the Grass instrument.[1]

As with the transducer previously described, the unit is placed in a glass tube (*E*), 3 cm. in diameter, which is attached to the top of the plethysmograph (*F*). In this instance it is necessary to use distilled water in the plethysmograph. As the height of the water column fluctuates, the resistance across the rods varies. These variations are reflected as a modulation of the carrier frequency.

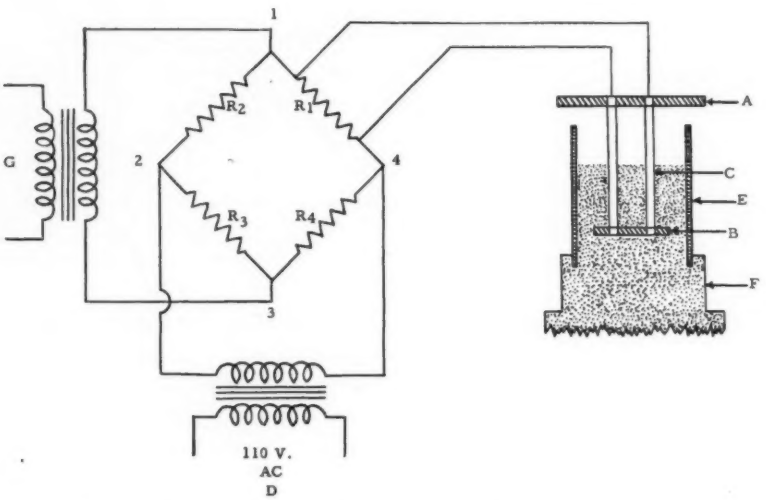


Fig. 3.—Transducer for use with a carrier-amplifier system.

Experience has shown that there is a length over the surface of the rods where perfectly linear calibration may be obtained. This has been found more than sufficient for plethysmographic work. Sample calibration curves with the addition of 1 ml. increments are shown in Fig. 4, *a* and *b*.

III. The third method which we have used utilizes the commercially available P23A Statham strain gauge (0 to 75 cm. pressure). The method is based on the principle of hydraulics in relating volume to pressure.

A glass tube (*T*) leading from the plethysmograph is provided with a side arm (*S*), as is shown in Fig. 5. The side arm is connected to the gauge, the output of which is then fed into a suitable amplifier and recorder. In such a system utilizing a homogeneous fluid and a glass tube of uniform diameter, the following relationships are evident:

1. $P = hdg$. where P = pressure, h = height of fluid column, d = density of fluid, and g = acceleration;
2. Assuming that d and g remain constant, then P is proportional to h;
3. $V = Ah$ where V = volume of fluid, A = cross-sectional area of tube (T), and h = height of fluid in tube.

Equating 2 and 4, one may see that the pressure recorded in the side arm, S, is proportionate to the height, h, of the fluid. Since P α h and v α h, then p α v when the cross-sectional area, density of fluid, and gravity remain constant. The only limiting factor is the uniformity of the cross-sectional area of tube which

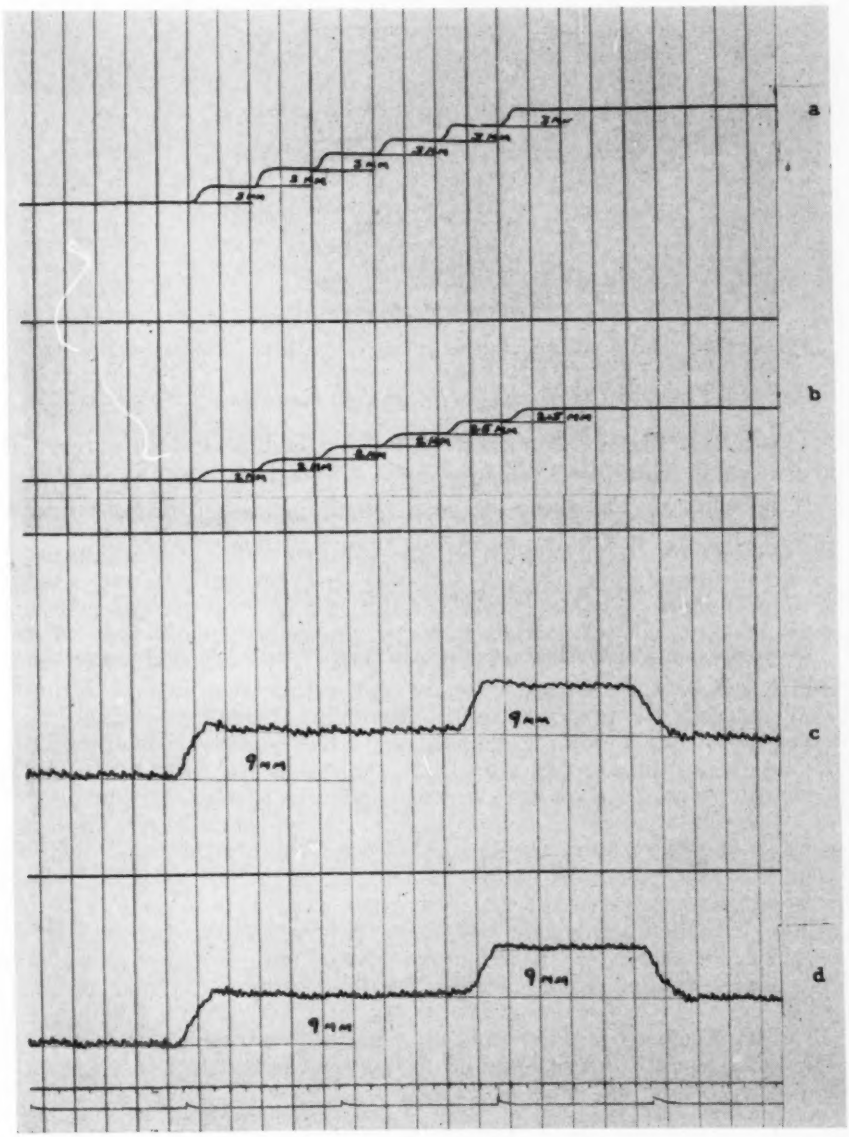


Fig. 4.—See text.

can be made constant to within 0.0002 inch. This method also has the advantage of electrical calibration, which can be performed with the Statham gauge and checked against known volume change. A record produced by adding 5 ml. volume of water to this system is shown in Fig. 4, *c* and *d*.

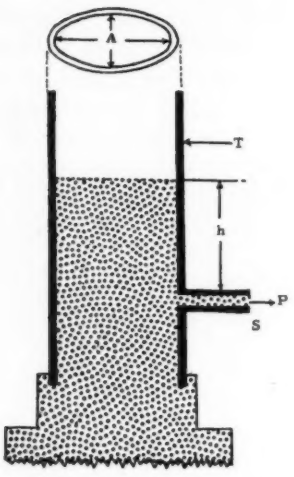


Fig. 5.—Diagramatic representation of system relating pressure to volume.

SUMMARY AND CONCLUSIONS

1. Three methods are described to make possible electronic recording of volume changes in water plethysmography.

2. It is believed that these methods are simple, sensitive, and linear, and that they should lead to greater use of this method of plethysmography.

REFERENCE

1. Simeone, F. A., Cranley, J. J., Grass, A. M., Linton, R. R., and Lynn, R. B.: An Oscillographic Plethysmograph Using a New Type of Transducer, Science 116: 355, 1952.

PROCEEDINGS OF THE CENTRAL SOCIETY FOR CLINICAL RESEARCH

Twenty-Ninth Annual Meeting

Chicago, Ill., Nov. 9 and 10, 1956

ABSTRACTS

70. THE EFFECT OF NOREPINEPHRINE ON SURVIVAL IN EXPERIMENTAL ACUTE HEMORRHAGIC HYPOTENSION

ROSS C. KORY, M.D., STEPHEN CLOSE, M.D., JOHN A. WAGNER, B.S., AND RALPH A. KLOEHN, JR., B.S., MILWAUKEE, WIS.

(INTRODUCED BY MAURICE HARDGROVE, M.D.)

A standard bleeding technique was developed in 37 dogs whereby it was possible to predict that the mean arterial blood pressure would be maintained at approximately 40 mm. of Hg for a 35-minute period. With this technique, a group of dogs was given norepinephrine during and for approximately 25 minutes after this standard bleeding procedure. The responses were compared with those in a similar group of control dogs given comparable quantities of dextrose solution. By using this "predictive" method, the blood pressure could be increased with norepinephrine during the latter part of the bleeding period with the assurance that without the addition of norepinephrine, the blood pressure would have remained at approximately 40 mm. of Hg as in the control animals.

In the first series, pentobarbital anesthesia was employed in 9 control dogs and 9 dogs given norepinephrine. Thirty-three per cent of the control dogs died as compared with 67 per cent of those receiving norepinephrine.

In the second series, morphine analgesia was employed in 20 control dogs and 17 dogs given norepinephrine. The mortality in the control group was 35 per cent as compared with a 59 per cent mortality in the group receiving norepinephrine.

In order to assess the effect of norepinephrine after termination of bleeding, the same experimental technique was employed in 32 additional dogs except that norepinephrine was not begun until after the 35-minute hypotensive period. For 16 dogs in the control group the mortality rate was 31 per cent. In the 16 animals receiving norepinephrine there was a 62 per cent mortality rate.

In addition to the increased mortality, earlier death was observed in dogs receiving norepinephrine. Autopsies in dogs dying during norepinephrine administration demonstrated marked visceral ischemia in striking contrast to the findings in the control dogs and in those animals dying some time after norepinephrine administration.

The results of this study indicate that under these particular experimental conditions, norepinephrine exerts a significantly detrimental effect ($P = < 0.01$ for the entire series) in acute hemorrhagic hypotension.

71. INTERACTION BETWEEN DESOXYRIBONUCLEIC ACID AND QUINACRINE: EFFECT ON DNASE SUSCEPTIBILITY

N. B. Kurnick, M.D., and I. E. Radcliffe (By Invitation), Long Beach, Calif.

Whereas the therapeutic value of quinacrine (atabrine) in systemic lupus erythematosus is doubtful, it has been shown to interfere with the L.E. cell phenomenon in vitro. Since the L.E. cell phenomenon involves desoxyribonucleic acid (DNA) and desoxyribonuclease (DNase), we have investigated the effect of quinacrine on this system.

We have found, by viscosimetric methods, that quinacrine forms a compound with polymerized DNA, which is not susceptible to DNase depolymerization. The suppression of depolymerization is not due to inhibition of the enzyme since the inhibition is independent of the enzyme-quinacrine concentration. It is dependent on the quinacrine-DNA ratio. The minimum quinacrine-DNA ratio at which inhibition is complete corresponds to the stoichiometry of the quinacrine-DNA compound as determined by dialysis, viscosimetry, and ultracentrifugation.

Quinacrine displaces methyl green from the methyl green-DNA compound and also prevents access of DNase to DNA. This suggests common sites of attachment.

Quinacrine apparently prevents the L.E. cell phenomenon, then, by reacting with the nuclear DNA so as to prevent depolymerization by DNase. This is quite different from the action of the specific intracellular protein inhibitor of DNase, which reacts with the enzyme and not the substrate. It may be that the former mechanism is deleterious to the cell and is related to the mechanism of its antiplasmodial action.

72. SERUM LIPOPROTEINS IN HEPATOBILIARY DISEASE

Daniel S. Kushner, M.D. (By Invitation), Alvin Dubin, M.S., (By Invitation), Gordon I. Fels, Ph.D. (By Invitation), and Hans Popper, M.D., Chicago, Ill.

Alterations of serum lipoproteins in hepatobiliary disease have been established by ultracentrifugation and some observations have been made by zone electrophoresis. To clarify these changes, the serum of 96 individuals was subjected to paper electrophoretic separation with the Durrham-type vertical cell. Proteins were stained with Bromphenol blue and lipids with Oil red 0. Values were expressed in per cent and in grams with the total lipid turbidity serving as reference. Patient material included 13 normal individuals, 19 with obstructive jaundice, 25 with viral hepatitis, and 39 with cirrhosis. Alterations of protein fractions were limited to elevation of the beta fraction in obstructive jaundice, and diminution of albumin and elevated gamma globulin which occurred regularly in cirrhosis and sometimes in hepatitis. Significant alterations of lipoprotein fractions included elevation of the beta fraction which was marked in obstructive jaundice, moderate in hepatitis, and slight in some cases of cirrhosis. The alpha lipoprotein fraction entirely and consistently disappeared at the height of obstructive jaundice. However, this was also the case in 4 patients with hepatitis, in 2 with nutritional cirrhosis, and in 2 with postnecrotic cirrhosis. The disappearance of alpha

lipoproteins could not be correlated with the degree of bilirubinemia, elevation of total lipids, elevation of phospholipids, or with the degree of liver cell damage. Possibly the alpha lipoprotein disappearance is related to an excess of substances, such as bile acids, which alter either the velocity of migration of the alpha fraction or its solubility or lipid-binding properties. Mobility of alpha lipoproteins could be altered by the addition of saponin to normal serum, resulting first in diminished velocity of migration of the alpha fraction, and then in its disappearance, presumably into the beta fraction, analogous to the known mobility alterations induced by heparin and lipositol.

73. IN VITRO AND IN VIVO STUDIES OF ACID-CITRATE-DEXTROSE-INOSINE (ACDI) AS A BLOOD PRESERVATIVE

ROBERT D. LANGE, MAJOR, MC, USAR, MARIO R. ESCOBAR, B.A., AND WILLIAM H. CROSBY, LIEUTENANT COLONEL, MC, USA, WASHINGTON, D. C.

(INTRODUCED BY DIETER KOCH-WESER, M.D.)

The addition of the purine nucleoside inosine to acid-citrate-dextrose (ACD) solution has been proposed as a method of prolonging the storage period of blood used for transfusion purposes. This study was undertaken to compare ACDI (1.8 Gm. inosine) and ACD as blood preservatives. For each study, 450 ml. of blood was stored in glass bottles or plastic packs for periods up to 42 days at 4° C. To make the comparison, biochemical and hematologic tests were done in vitro and the survival of 20 ml. of Cr^{51}-tagged red cells was measured in vivo. Forty-three studies were performed.

Other than a higher plasma hemoglobin in units collected by vacuum in glass, the type of container did not affect the results.

On storage, the expected fall in Na, and rise in K and hemoglobin occurred in the plasma. Inosine did not retard these changes.

The erythrocytes stored in ACD showed at least 70 per cent survival at 24 hours after 21 days of storage and in ACDI after 28 days of storage. However, when stored for 35 and 42 days in ACDI, 8 of 17 units failed to show 70 per cent survival at 24 hours. In general, there was little loss of nonviable cells at 30 minutes after transfusion and the $T\frac{1}{2}$ of viable cells after 28 days of storage was within normal range. However, the $T\frac{1}{2}$ decreased as the storage time increased.

In four studies 450 ml. of ACDI blood was transfused. Pulse rate and blood pressure were not affected. However, in two instances where uric acid excretion was measured, it was found to be slightly increased.

Under the conditions of these studies it was shown that the addition of 1.8 Gm. of inosine to ACD prolonged the storage period of blood for seven days. At longer periods of storage the results were variable.

74. THE EFFECT OF CORTICOTROPIN UPON SERUM PROTEINS, CREATININE CLEARANCE, AND URINARY ELECTROLYTE EXCRETION IN DIABETIC INTERCAPILLARY GLOMERULOSCLEROSIS

JOHN A. LAYNE, M.D., GREAT FALLS, MONT.

The improvement which has followed the use of corticotropin in the treatment of edema of congestive heart failure and edema accompanying the nephrotic syndrome led us to use this hormone in the treatment of a patient having the Kimmelstiel-Wilson syndrome with recurrent, moderately resistant, and extensive edema. A complete metabolic study was carried out

prior to, during, and after the intramuscular administration of 100 mg. of corticotropin daily for 8 days. The patient's serum was analyzed for total protein by the biuret method; fractionation was accomplished using filter paper electrophoresis and quantitative estimations performed using the Spinco analytrol. The study also included daily creatinine clearance determinations, the measurement of urinary and plasma electrolytes, and other pertinent blood chemical studies.

Although the patient gained in weight and his edema increased immediately following the administration of corticotropin, his weight at the end of the 8-day period of administration was less than when the hormone was started. As expected, he was in positive sodium balance during the 8-day period of administration of corticotropin. Immediately following discontinuance of corticotropin his sodium balance became negative and remained negative for more than 3 weeks, during which time there was slow but consistent weight loss and clearing of edema. Significant alteration in total serum protein or its fractions did not occur, nor was there significant improvement in creatinine clearance, during or following the period of corticotropin administration. Although the patient subsequently expired 3 months later, he remained free of edema until death. Proteinuria was not significantly altered during or following administration of corticotropin.

75. A COMPARISON OF THE EFFECT OF TWO ANTIBIOTICS (NOVOBIOCIN AND SPIRAMYCIN) USED IN COMBINATION WITH THAT OF SPIRAMYCIN ALONE ON THE ANTIBIOTIC SENSITIVITY OF MICROCOCCUS PYOGENES

MARK H. LEPPER, M.D., HARRY F. DOWLING, M.D., GEORGE G. JACKSON, M.D., HAROLD W. SPIES, M.D. (BY INVITATION), AND MARGARET MELLODY, M.S. (BY INVITATION), CHICAGO, ILL.

The rapid increase in resistant strains of staphylococci which occurs following the introduction of an antibiotic has been shown to be caused primarily by the spread of strains which were resistant to the antibiotic before its use had been begun. It has been postulated that the occurrence of this phenomenon could be minimized if two effective antibiotics were used simultaneously.

The availability of Novobiocin and Spiramycin, at the same time, therefore presented the opportunity to study the effectiveness of combination therapy on the mitigation of the spread of resistant forms.

Since January, 1956, all patients, with rare exception, in the Municipal Contagious Disease Hospital of Chicago who required antibiotic treatment received Spiramycin in a dose of 100 mg./kilo/24 hours and Novobiocin in a dose of 30 mg./kilo/24 hours.

For the coagulase-positive isolates obtained from admission cultures, the percentage of strains resistant to 1 mcg./ml. or more of Novobiocin was: December, 6.2; January, 7.2; February, 0.0; March, 0.0; April, 6.7; May, 0.0; and June, 0.0. However, at the time of the follow-up cultures in the hospital, the percentages were: December, 0.0; January, 2.4; February, 16.7; March, 19.1; April, 17.1; May, 32.1; and June, 29.8.

The percentage of coagulase-positive isolates resistant to 10 mcg./ml. or more of Spiramycin on the admission cultures were: December, 3.0 and in January through June, zero. But among the follow-up isolates, the percentages were: December, 0.0; January, 2.7; February, 8.9; March, 14.2; April, 23.1; May, 15.5; and June, 16.0.

Among the coagulase-positive isolates, the percentage of strains resistant to 10 mcg./ml. of erythromycin increased from 12 to 20 per cent by March.

When Spiramycin was used alone in a similar study six months before the combination was used, there was a rapid increase in the percentage of strains resistant to 10 mcg./ml. of Spiramycin. Within one month these increased from a pre-experimental level of 4.0 per cent to 26.5 per cent. The percentage of strains resistant to 10 mcg./ml. erythromycin similarly rose from 10 per cent to 33 per cent in the first month.

76. THE UPTAKE, FATE, AND EXCRETION OF SULFUR^{35}-LABELED L-METHIONINE IN THE NORMAL RAT

WILLIAM C. LEVIN, M.D., JOHN E. PERRY, M.D. (BY INVITATION), AND T. G. BLOCKER, JR., M.D. (BY INVITATION), GALVESTON, TEX.

Sulfur^{35}-labeled L-methionine was administered intravenously to a series of rats weighing 200 to 250 grams. Urine and feces were collected quantitatively. The rats were exsanguinated at different intervals and the organs were analyzed for S^{35} content by measuring the extracted sulfur as barium sulfate. In all animals a peak of activity in the organs was attained within a few hours. The activity then slowly decreased to reach an equilibrium. The highest uptakes expressed as the percentage of the administered dose taken up by each organ were found in the liver, skin, small intestine, plasma, pancreas, large intestine, kidney, and bone, in decreasing order. The spleen, ovaries, and testes showed very low uptakes and the erythrocytes displayed a slowly rising uptake.

The uptake of radioactivity expressed as the percentage of the administered dose per gram of organ was highest in the pancreas, kidney, liver, small intestine, large intestine, and plasma, in decreasing order. The urinary excretion was very rapid during the first 24 hours and then decreased by a variable amount. The fecal excretion was very low in most of the animals, almost never rising above 5 per cent of the administered dose.

This technique is being applied to the study of the effect of burns on the rate and amount of incorporation of L-methionine by the various organ systems.

77. FEVER AND INFECTION IN LEUKEMIA

JOHN LOUIS, M.D. (BY INVITATION), LOUIS R. LIMARZI, M.D., AND M. H. LEPPER, M.D., CHICAGO, ILL.

The occurrence of fever in leukemia is usually thought of as a manifestation of the disease. The frequency fever as associated with infection is minimized by most physicians. This has been particularly true since the advent of antibiotics. Routine use of antibiotics has introduced another complication, that of superinfection.

The course of 41 patients with leukemia (32 acute and 9 chronic) was reviewed. Nineteen were male patients and 22 were female patients with ages ranging from 6 months to 63 years. These patients were divided into four groups: 14 died because of infection, Group I; 11 died with an associated infection, Group II; 9 died without evidence of infection, Group III; and in 6 the presence of infection was not determined, Group IV. The mean duration of life was shortest in Group IV (2.5 months) and longest in Group I (8.4 months).

Bacterial infection could be demonstrated in 22 out of 26 patients who had fever shortly before death.

There were 82 febrile episodes during the course of the disease in this series of patients. Bacterial cultures were not obtained in 30 episodes. Fever subsided in 7 episodes with and 10 episodes without antibiotic therapy. Bacterial cultures were obtained in 42 and were positive in 38 febrile episodes. Febrile remissions occurred in 12 episodes (57 per cent) of those who received antibiotic therapy based upon identification of the infecting organism. Favorable response occurred in 8 episodes (19 per cent) following administration of antibiotics without bacteriologic studies. Improvement without use of antibiotics occurred in 9 episodes (47 per cent).

The most frequent sites were the blood, respiratory tract, and ear. All patients in Group I had septicemia. *Micrococcus pyocyaneus, Pseudomonas aerogenes,* and hemolytic *Endamoeba coli* in this order of frequency were the most common organisms found. The occurrence of infection could not be significantly correlated with the white blood count, degree of remission, or type of therapy.

In our experience infection appears to be the primary cause of fever in leukemia. In spite of this problem the judicious use of antibiotics may possibly prolong the life of the leukemic patient in the future.

78. CORRECTION OF A MAJOR ERROR IN DETERMINING URINARY PORTER-SILBER REACTIVE CORTICOSTEROIDS IN THE PRESENCE OF GLYCOSURIA

LAWRENCE H. LOUIS, SC.D. (BY INVITATION), PAUL A. EILER, B.S. (BY INVITATION), DAVID H. P. STREETEN, M.R.C.P., AND JEROME W. CONN, M.D.,
ANN ARBOR, MICH.

Urinary "17-hydroxycorticosteroids" (17-OHCS) reflect the level of adrenal "glucocorticoid" production. The latter, when secreted in excess, promotes hyperglycemia and glycosuria. Persistently abnormal elevation of urinary 17-OHCS together with diabetes mellitus suggests cause and effect and warrants the diagnosis of Cushing's syndrome. We have discovered a technical error which, if unrecognized, will continue to result in erroneous interpretations and diagnoses.

When glucose-containing urine of diabetic patients is incubated with β-glucuronidase at pH 6.5 in the determination of 17, 21-dihydroxy-20-ketosteroids (17-OHCS) by the method of Silber and Porter, there results: (1) considerable fermentation of the sample, and (2) values for 17-OHCS much higher than normal. Further study of these observations have disclosed the following additional facts:

1. Addition of glucose to urine of normal people results in great elevation of the apparent 17-OHCS value.
2. It is not the glucose molecule which produces this effect but a degradation product of glucose occurring during enzymatic hydrolysis of the urine sample.
3. This material is soluble in chloroform and reacts with phenylhydrazine to give a non-17-OHCS chromogen; thus falsely high values for 17-OHCS.
4. Addition of sodium bisulfite to the urine-enzyme-glucose mixture before incubation eliminates selectively the interfering material.
5. Bisulfite added to aglycosuric urine has no effect.
6. Bisulfite added to hydrocortisone (free) solution has no significant effect.

It is likely that the interfering material is an aldehyde and/or a ketone.

Whenever glycosuric specimens are run for 17-OHCS by the excellent and popular method of Silber and Porter, the specific modification to be described should be employed.

79. ESTIMATION OF THE RATES OF UPTAKE OF RB^{86} BY THE HEART, LIVER, AND SKELETAL MUSCLE OF PATIENTS WITH AND WITHOUT CARDIAC DISEASE

W. D. Love, M.D., and G. E. Burch, M.D., New Orleans, La.

Rates of Rb^{86} uptake by different tissues and organs have been estimated by external monitoring in 35 patients. Similar methods for determining myocardial Rb^{86} uptake in dogs had an error of ±20 per cent.

The Rb^{86} concentration of arterial plasma was maintained nearly constant for 35 minutes by infusing a total of 0.2 mc. Rb^{86} at a progressively decreasing rate. Radioactivity over the precordium, upper thorax, liver, and thigh was measured using a NaI crystal collimated with 12.5 cm. of lead. Areas were remonitored 24 hours later when near equilibration of tissues and plasma had occurred.

Treating Rb^{86} as a tracer of potassium and each area as a single compartment exchanging with the plasma, rates of Rb^{86} uptake were expressed as per cent of the regional potassium exchanged per minute with plasma potassium. In 16 normal patients these were: precordium 0.57 ± 0.14, upper thorax 0.29 ± 0.07, liver 0.36 ± 0.16, and thigh 0.10 ± 0.03.

Nitroglycerin (1.3 mg.) administered sublingually did not affect the rate of rise of precordial radioactivity.

When myocardial radioactivity was considered to be equal to precordial radioactivity minus the radioactivity over the upper thorax, the estimate of apparent myocardial potassium exchange in normals was 1.9 ± 1.0 per cent per minute. This rate would require a coronary blood flow of approximately 90 ml. per 100 Gm. myocardium per minute if, as in the dog, two-thirds of the plasma Rb^{86} was removed during passage of the blood through the myocardium.

In seven patients with angina pectoris the standard injection produced arterial plasma Rb^{86} concentrations averaging 57 per cent higher than the normals and 28 per cent greater than twelve patients with other forms of cardiac disease. This low rate of loss of Rb^{86} from the plasma may reflect reduced blood flow to the tissues, associated with diminished cardiac output. Rb^{86} uptake in individual areas was from 20 to 45 per cent slower in these patients with angina pectoris than in patients without cardiac disease.

These methods of estimating the effective Rb^{86} exchange by tissues in intact patients may have value, in part because of the probable relationship of exchange and blood flow.

80. THE OXYGEN CONSUMPTION OF THE NONWORKING HEART

William P. Mc Keever, Captain, MC, USAR, Philip C. Canney, Major, MC, USA, and Donald E. Gregg, M.D., Ph.D., Washington, D. C., and Chicago, Ill.

(Introduced by Craig W. Borden, M.D.)

While there is much information concerning the metabolism of resting skeletal muscle, technical difficulties have prevented similar studies of mammalian cardiac muscle in situ. To obtain this information, it is necessary to determine cardiac oxygen consumption continuously and furthermore to pro-

duce a condition in which cardiac work can be repeatedly reduced to zero and back to control levels. This has been accomplished in the anesthetized open-chest dog by perfusing the left main coronary artery and draining coronary sinus blood to the exterior. Portions of the coronary arterial and sinus blood streams were diverted through two photoelectric densitometers to detect changes in oxygen saturation. Thus a continuous record of cardiac oxygen usage was obtained. To reduce external cardiac work to zero several procedures were studied. They included rapid exsanguination by suction and cardiac arrest produced by vagal stimulation or intracoronary infusion of potassium chloride. The data from all experiments for nonworking oxygen usage range from 0.7 to 4.8 ml. oxygen per 100 Gm. myocardium per minute. Expressed as a percentage of prior working levels the range is 16-40 per cent. This range was considerably narrowed where it was possible to study all procedures in the same animal.

Cardiac efficiency is defined as the ratio of measured work to the energy output due to work. The denominator is obtained by subtracting resting from total oxygen usage. Since the nonworking oxygen usage is a significant portion of the total usage, its neglect in measurements of efficiency will introduce considerable error. Norepinephrine appears to increase total and nonworking oxygen utilization disproportionately to work output, thereby reducing cardiac efficiency.

81. LIVER UPTAKE AND BLOOD CLEARANCE STUDIES OF RADIOACTIVE (I^{131}) ROSE-BENGAL

WM. MARSHALL, M.D. (BY INVITATION), AND DONALD D. KOZOLL, M.D., CHICAGO, ILL.

Radioactive Rose-Bengal resembles bromsulfalein in measuring the circulatory volume of the liver, hepatocellular function, and biliary patency. Because of minute dosage, it can be used in the differential diagnosis of jaundice. This report deals with the uptake of this material in 41 normal hospital controls and 60 clinical cases of jaundice.

Five microcurie doses of isotope were given intravenously. A wide-angle counter was placed over the liver and attached to a rate meter and an Esterline Angus Recorder, and graphic records were obtained for the first ninety minutes and then at 6- and 24-hour intervals. Concurrent blood samples were drawn and measured in a deep-well counter. Peak liver uptake was calculated from the graphic records, and then divided by the time required to achieve this peak as rate of uptake.

In 41 controls the peak concentration over the liver averaged 21,500 counts per minute (c.p.m.). At 6 hours it was 7,250 c.p.m. and at 24 hours 3,750 c.p.m. The lowest peak concentration of the liver appeared in 25 cases of cirrhosis (12,250 c.p.m.) which returned to almost control levels at 6 and 24 hours. Carcinomatous extrahepatic obstruction (12 cases) achieved a peak count of 13,000 c.p.m., but at 6 and 24 hours remained markedly elevated. In infectious hepatitis (17 cases) and calculus extrahepatic obstruction (7 cases), peak hepatic concentrations and clearance from the liver were markedly alike and returned to almost normal levels by 6 and 24 hours.

The *rate of liver uptake* in the normal averaged 689 c.p.m. (range 525-980). The slowest rate of uptake (249 c.p.m.^{2}) was malignant obstruction; cirrhosis averaged 274 c.p.m.^{2}. Calculus obstructions averaged 353 c.p.m.^{2}, and hepatitis 479 c.p.m.^{2}. All liver clearance rates were slower than normal at 6- and 24-hour intervals.

Peak blood counts showed the greatest values in malignant construction (174 c.p.m.), then cirrhosis (102 c.p.m.) and hepatitis (92 c.p.m.), whereas

calculus obstruction (41 c.p.m.) was close to that of normal blood levels (43 c.p.m.). At 6 hours the blood level was highest in malignant obstruction and approximately the same in all other jaundiced cases. At the end of 24 hours all levels approached normal except the carcinomas which were still markedly elevated.

82. THE ACUTE CALCIFICATION OF DAMAGED MUSCLE, WITH PARTICULAR REFERENCE TO ACUTE POSTTRAUMATIC RENAL INSUFFICIENCY

W. H. Meroney, M.D., G. K. Arney, M.D., W. E. Segar, M.D., and H. H. Balch, M.D., Washington, D. C.

(Introduced by A. C. Corcoran, M.D.)

Previous studies demonstrated that serum calcium and phosphorus concentrations were inversely related in patients with posttraumatic renal insufficiency and that the deviations from normal were most severe in those patients with greatest damage to muscle. Infusions of calcium caused only transient elevations of serum concentration, and the rate at which infused calcium escaped from the serum appeared to be proportional to the degree of hyperphosphatemia, which, in turn, was proportional to the degree of muscle damage.

These relationships suggested that the fate of the infused calcium was deposition in the damaged tissue, a phenomenon well recognized in chronic states but not known to occur acutely. The clinical interest in this possibility stems from a reluctance to treat potassium intoxication with massive calcium infusions because the calcium might be harmful if deposited in viscera.

To test this possibility dogs were given tracer doses of $Ca^{45}Cl_2$ intravenously on Day 0, and plasma samples collected daily thereafter were analyzed for radioactivity and chemical concentration. On Day 3 bilateral nephrectomies were performed, and the right hind-limb muscles were crushed mechanically without damaging the bones. On Day 6 muscle biopsies were taken from symmetrical areas of the crushed and uncrushed hind limbs. The crushed muscle specimens contained some 30 times as much radioactivity and 12 times as much chemically-measured calcium as the respective controls. Comparison of the specific activity (counts/min./mg. calcium) of crushed muscle with the specific activity of plasma revealed that the calcium was deposited in the muscle immediately following trauma and little further exchange with plasma occurred. The magnitude of calcium deposition was such that 1 Kg. of crushed muscle would have accepted more calcium than that contained in 100 ml. 10 per cent calcium gluconate, which is a generous dose for the treatment of potassium intoxication.

Similar studies in nonnephrectomized dogs revealed only twice as much calcium in the crushed muscle specimens as in the controls. The administration of phosphate, acid, alkali, and parathyroid hormone failed to alter the twofold increase in calcium content of crushed muscle in nonnephrectomized dogs.

83. TOBACCO SMOKING, MOTOR EXHAUST FUMES, AND GENERAL AIR POLLUTION IN RELATION TO LUNG CANCER INCIDENCE

C. A. Mills, M.D., Cincinnati, Ohio

In evaluating, singly and in combination, the three most strongly suspected factors in lung cancer etiology, we organized a survey covering 3 con-

trasting white populations—persons living in Cincinnati's dirtier basin districts, those in the city's cleaner suburbs, and rural populations living in Ohio's least urbanized counties. The survey covered sex, age, place and duration of residence, occupation, tobacco-smoking habits, and estimates of annual motor-travel mileage. Living population surveys included house-to-house visitation in the basin districts and a combination of house visitation and telephone calls in the suburban and country districts. Final data tabulations and rate calculations included only those with 10 years' residence or more in the given area (discarding only about 5% of total surveyed numbers); it thus seemed justifiable to project long-term-residence surveyed numbers proportionately upon total white area populations (1950 census), in order to secure numbers at risk and death rates in each population category, age 40 and over.

Similar information on area residents dying of lung cancer during the 9-year period (1947-1955) was obtained directly from the death certificates from hospital records, and from near relatives or close friends.

Under standard conditions of age and air pollution other than tobacco smoke, we found pipe-cigar smoking in white men to be associated with a two- to threefold increase in lung cancer (above nonsmoker rates), light cigarette smoking (1 to 14/day) with a somewhat lesser increase, moderate cigarette smoking (15 to 35/day) with a four- to sixfold increase, and heavy cigarette smoking with a ten- to twentyfold increase. Exposures to annual urban traffic mileage over 12,000 miles were associated with a further two- to threefold increase in lung cancer hazard for men of all smoking categories (not for nonsmokers), while additional exposure to general urban air pollution was associated with a further doubling of hazards already existing in association with the other 2 factors. Extensive motor travel in dominately rural areas carried no additional lung cancer hazards. Nonsmoking men of all areas exhibited no significant difference from the low female lung cancer rates found, regardless of motor exhaust or general urban air pollution exposure.

84. RESPONSE OF THE IRON-TRANSPORT SYSTEM TO THE INGESTION OF FERROUS SULFATE

J. H. Mitchell, M.D. (By Invitation), E. R. Halden, M.D. (By Invitation), S. Bryan (By Invitation), and E. E. Muirhead, M.D., Dallas, Tex.

To elucidate the response of the iron-transport system following the ingestion of ferrous sulfate, the serum iron concentration, unsaturated iron-binding capacity, and total iron-binding capacity were followed in 14 patients with iron-deficiency anemia and in 6 normal subjects. Blood samples were drawn prior to and at 1, 2, 4, 6, and 8 hours after the ingestion of 1.33 Gm. of ferrous sulfate. Serum iron was determined by the method of Barkan and Walker; unsaturated iron-binding capacity was determined as described by Schade and Caroline; total iron-binding capacity was obtained by summation of the values for serum iron and the unsaturated iron-binding capacity.

In the patients with iron-deficiency anemia the total iron-binding capacity decreased from a value of 375 ± 57 μg to 221 ± 35 μg from 2-6 hours after the ingestion of iron. Throughout this period the serum iron level rose while that of the unsaturated iron-binding capacity fell to a greater extent. In 5 anemic patients the encroachment was complete with the iron-transport system totally saturated at a significantly lower level of total iron-binding capacity. In normal subjects the same phenomenon occurred to a much lesser degree. At eight hours the total iron-binding capacity in both groups approached control levels.

These observations indicate that, during the absorption of iron from the intestines in normal and iron-deficient subjects, the total iron-binding capacity of the plasma (transport system) is depressed. This depression is particularly prominent in iron-deficiency states. The lowered iron-binding capacity suggests sequestration of transferrin in regions where iron is absorbed, utilized, and stored. The greater decrement of transferrin in the anemic patients may be attributed to a more rapid turnover and/or sequestration of the iron-protein complex.

85. THE EFFECT OF BLOOD PRESSURE CONTROL ON THE RENAL VASCULAR DETERIORATION ASSOCIATED WITH HYPERTENSION

JOHN H. MOYER, M.D., KEITH PEVEY (BY INVITATION),* W. O. AUTERY (BY INVITATION),* AND RALPH FORD, M.D. (BY INVITATION), HOUSTON, TEX.

Although there is no good method for assessing the general clinical status of patients with hypertensive vascular disease in absolute numerical terms, such an evaluation is possible relative to some specific vascular beds if blood flow and functional capacity are used to express the status of these areas. This is particularly true of the kidneys since renal blood flow and glomerular filtration rate can be measured and expressed in absolute values. Furthermore, the renal vasculature is probably more vulnerable to the renal vascular deterioration associated with hypertension than is any other system in the body. For these reasons, an evaluation of renal function was undertaken in order to determine the effects of antihypertensive therapy in 2 groups of patients with hypertensive vascular disease, i.e., a control group and a treated group. The treated group consisted of those patients who followed an effective treatment program for 2 years or more, whereas the control group did not follow an effective program for one reason or another. The severity of disease as estimated by clinical evaluation and the degree of renal vascular damage prior to therapy were about the same in both groups of patients. Inulin and para-aminohippurate were used to determine glomerular filtration rate and renal blood flow, respectively. The observations indicate that after 2 years, those patients who were not treated adequately fell into 2 groups: (1) those patients with mild disease showing very little renal vascular deterioration, and (2) those with severe disease showing marked deterioration. All ineffectively treated patients with malignant disease were dead. In the effectively treated group, progressive vascular deterioration did not occur. Although the vascular deterioration was arrested, renal function did not return to normal. Patients with severe azotemia and uremia died despite therapy. The results indicate that effective antihypertensive therapy can be expected to arrest the progress of renal vascular damage resulting from hypertension, but the degree of damage that exists at the time therapy is instituted is more or less permanent and probably is not reversible.

86. METABOLIC AND PHYSIOLOGIC STUDIES OF TWO CASES OF PRIMARY ALDOSTERONISM

ROBERT C. MUEHRCKE, M.D., AND MALCOLM D. MILNE, M.D., M.R.C.P., CHICAGO, ILL., AND LONDON, ENGLAND

(INTRODUCED BY FORD K. HICK, PH.D., M.D.)

Two women, aged 44 and 56 years, with primary aldosteronism had metabolic and physiologic studies. These investigations were made during three

*Senior Medical Students.

periods: (1) before potassium replacement therapy, (2) while taking potassium supplements, and (3) after removal of an aldosterone-secreting adenoma. The patients differed widely with regard to severity of disease, mode of presentation, and clinical state. Patient 1 had severe symptoms of intermittent muscular paralysis for twelve years. Her metabolic disorder was modified by the presence of severe irreversible renal disease. Patient 2 was asymptomatic and had no histologic abnormalities of the kidneys.

Both patients had: (1) moderate hypertension with cardiac enlargement, (2) hypokalemic alkalosis, (3) increased loss of urinary potassium, calcium, and magnesium, (4) sodium retention, and (5) renal impairment as evident by proteinuria, failure to concentrate the urine, and reduction of creatinine clearances.

On 200 meq. potassium chloride per day the following observations were made: (1) the patients were found deficient of 1,000 meq. and 400 meq. potassium, respectively; (2) the serum alkalosis subsided; (3) the serum potassium level approached normal; (4) the urinary excretion of alpha ketoglutamic acid and citric acid increased; (5) the isosthenuria persisted; and (6) *the neutral or faintly alkaline urine, previously described in primary aldosteronism, was found to be the result of potassium deficiency rather than the specific effect of the aldosterone-secreting tumor.* In both patients, electrolyte analyses were made of muscle removed during adrenalectomy. These studies indicated a moderate reduction of muscle potassium and an elevation of muscle sodium: surgical renal biopsy studies failed to disclose tubular changes of "hypokalemic nephropathy."

Postoperative balance studies of both patients revealed: (1) a slight retention of potassium, (2) a definite retention of calcium and magnesium; (3) decrease in proteinuria and improvement of renal function. In the milder case with normal renal histology there was a normal postoperative retention of sodium and chloride despite removal of the aldosterone-excreting tumor. Several months postadrenalectomy, the blood pressure of both patients was returning to preoperative levels.

87. OBSERVATIONS ON THE DISAPPEARANCE FROM HUMAN BLOOD OF INTRAVENOUSLY ADMINISTERED FAT EMULSIONS

JOHN F. MUELLER, M.D., JACK M. IACONO, PH.D. (BY INVITATION), AND WILLIAM W. CLELAND, PH.D. (BY INVITATION), DENVER, COLO.

Recent advances in the preparation of a fat emulsion for intravenous administration to human beings have resulted in a product almost devoid of adverse reactions except fever, which occurs as a delayed phenomenon in approximately 25 per cent of initial infusions. Attempts to explain the febrile response on the basis of particle size, decomposition products, pyrogens, or in vivo hemolysis have all failed.

During the course of investigations into the role that mechanisms for transport and utilization of intravenously administered fat might have on the thermogenic response, a "clearing test" was devised which consists of optical density measurements of the recipient's serum before, at the end of, and 8 hours after the beginning of a constant rate infusion of 600 ml. of emulsified fat. One hundred sixty-eight such "clearing tests" have been performed in a heterogenous group of hospitalized and normal subjects. There is great

variation in the rapidity with which blood is cleared of the fat. In normal and moderately ill patients the clearing is slower than in the more severely ill subjects, with the prominent exception of those patients with advanced liver disease. The striking observation was that there is a highly significant positive correlation between the rapidity of clearing and the temperature elevation. These data suggest that in the sicker patients the blood is more rapidly cleared and fever develops. The mechanism of the febrile response might be endogenous heat production from rapid oxidation of fat within the cell in a person whose heat regulatory mechanisms are impaired by the primary illness, or it might conceivably be due to the release of a foreign material associated with the fat during the process of rapid clearing. The latter seems unlikely because of the observed thermogenic effect of emulsions utilizing purified oils.

Assays of the recipient's blood for "clearing factor activity" after a fat infusion reveals that the amount of fat removed from the blood in a given individual cannot be accounted for by "clearing factor activity" and that a large portion of the fat which is cleared must enter parenchymal and reticuloendothelial cells directly.

88. THE EFFECT OF BROMINE ION ON I^{131} THYROID FUNCTION STUDIES

Richard E. Peterson, M.D., and Masa Yamamoto, M.D., Iowa City, Iowa

(Introduced by Walter M. Kirkendall, M.D.)

The observation that a clinically hyperthyroid patient had normal I^{131} tracer studies when first studied after 6 weeks of 3 Gm. daily of Elixir Triple Bromides (E. T. Br.) aroused interest in the possibility of a bromine effect on radioiodine diagnostic studies.

Another hyperthyroid patient was studied with a tracer dose of Br^{82}. The thyroid concentrated 17-20 per cent of the Br^{82} tracer dose and 55-70 per cent of I^{131} tracer doses. At 24 hours, this patient had 57 per cent of the serum I^{131} organically bound (butanol extractable), while an insignificant amount (less than 0.4 per cent) of the serum Br^{82} was butanol extractable.

Twenty-five euthyroid patients were studied with I^{131} tracer studies before and on the third day of receiving E. T. Br. grams one q.i.d. The PBI was not altered significantly. At one hour, the thyroid uptake of iodine was depressed by 2 per cent of the dose ($P<0.01$) and the twenty-fourth-hour thyroid uptake was decreased 4.8 per cent of the dose ($P<0.1$). The 24-hour urine excretion of I^{131} was decreased 7 per cent of the dose with $P<0.05$. I^{131} counts over the calf (body background) were increased following the E. T. Br., averaging 35 per cent increase at 6 hours and 18 per cent increase at 24 hours. The total serum level of I^{131} and of butanol extractable I^{131} were slightly, but insignificantly, decreased.

These data imply the thyroid significantly concentrates but does not organically bind the bromine ion. Bromide in the dosage given depresses thyroid I^{131} uptake probably through competition in the halogen trapping mechanism. The decrease in urine excretion of I^{131} without change in the serum level of I^{131} is compatible with the increased extrathyroidal I^{131} concentration indicated by the increase counts over the calf following bromide administration. The possibility of E. T. Br. administration interfering with I^{131} thyroid diagnostic studies must be kept in mind.

89. VARIATION IN POTENCY OF ANTIGLOBULIN SERA PREPARED FROM SERUM CONTAINING WARM AND COLD AGGLUTININS

ANTHONY V. PISCIOTTA, M.D., AND JEAN E. HINZ (BY INVITATION), MILWAUKEE, WIS.

Some differences in properties between cold and warm agglutinins are known. These include variation in optimal temperature required for agglutination, neutralization of the antiglobulin reaction in pure gamma globulin in warm, but not cold, agglutination (Dacie, J. V.: Lancet **II**: 954, 1951), and the demonstration of agglutination in greater dilutions of antiglobulin serum of erythrocytes coated with "warm" antibodies (Dacie, J. V.: Nature **166**: 36, 1950; and Von Loghem and co-workers: Rev. Hemat. **6**: 286, 1951). Differential absorption studies using erythrocytes coated with warm and cold antibodies suggest specificity of the antiglobulin reaction in detecting these types of immune bodies (Crawford and Mollison: Lancet **II**: 955, 1951; and Renton, P. H.: Nature **169**: 329, 1952). The purpose of this study was to provide further evidence of serologic and antigenic differences between cold and warm hemagglutinins by preparing antiglobulin sera in the rabbit from human serum containing these antibodies.

Antiglobulin serum was prepared, using serum obtained from six patients with autoimmune hemolytic disease. Two of these patients had a potent cold agglutinin, two a warm agglutinin, two had a positive antiglobulin reaction with no free antibody in the serum. Antiglobulin serum was also prepared from pure gamma globulin and for controls from normal serum.

This battery of antiglobulin sera was tested against the erythrocytes of patients with warm and cold types autoimmune hemolytic anemia.

Antiglobulin serum produced from the two specimens containing warm antibodies had a greater affinity for erythrocytes coated with warm antibodies than those coated with cold antibodies. Also, "warm" antibody sera provoked a greater antigenic response in rabbits than "cold" antibody sera. On the other hand, anticold agglutinin serum showed slightly increased comparative activity against erythrocytes coated with cold hemagglutinins, but diminished titers with warm antibody-coated red cells. Over-all, the affinity of erythrocytes from patients with cold agglutinins for antiglobulin serum was lower than that from patients with warm antibody hemolytic anemia.

Results of cross-absorption experiments with warm and cold agglutinin-sensitized erythrocytes will also be reported.

90. NEPHROTIC SYNDROME IN ADULTS

VICTOR E. POLLAK, M.B., M.R.C.P.E. (BY INVITATION), ROBERT M. KARK, F.R.C.P., F.A.C.P., CONRAD L. PIRANI, M.D. (BY INVITATION), AND ROBERT C. MUEHRCKE, M.D. (BY INVITATION), CHICAGO, ILL.

In adults the nephrotic syndrome may be the clinical expression of many different disease states. It may result from damage primarily to glomeruli, from damage primarily to tubules, or from increased pressure in the renal veins, as in renal vein thrombosis and constrictive pericarditis. Clinical differentiation of these causes is difficult. We have studied by percutaneous renal biopsy, seventy-three adults with the nephrotic syndrome, and these studies have reflected a wide range of pathology and etiology.

Subacute or chronic glomerulonephritis was found twenty-six times. The lesions were membranous in some, proliferative in others, but mixed lesions were frequently found. Sixteen patients with diabetes mellitus had

the nephrotic syndrome. Diffuse glomerulosclerosis was found in three, diffuse and nodular lesions in seven, and diffuse, nodular, and exudative lesions in six. Eight patients with SLE had the nephrotic syndrome, and three others had the pseudonephrotic syndrome. The lesions of lupus nephritis were more severe in the pseudonephrotic group.

Primary renal amyloidosis was found once, bilateral renal vein thrombosis twice, and constrictive pericarditis once. One case had multiple myelomatosis without amyloid but with much Bence-Jones proteinuria and another appeared to be due to mercurial diuretics. Three patients had transient nephrotic syndrome with severe arteriolar nephrosclerosis, and in one patient the lesion was undiagnosed. In four patients studied only after diuresis the kidneys were normal. With increasing experience it was possible to make exact histologic diagnoses in over 90 per cent of cases, and to forecast, on the degree and type of glomerular damage, the prognosis and response to treatment with ACTH or corticosteroids.

The histologic appearance of the kidneys was unique in six patients. The glomerular capillaries contained an unusually large number of erythrocytes, but the basement membrane and epithelial and endothelial cells of the glomerulus were normal. Severe tubular degeneration and edema of the interstitial tissue were observed. ACTH or corticosteroid administration resulted in an excellent diuresis. During diuresis the tubular epithelium was taller and less degenerated, and the glomerular capillaries contained few erythrocytes. These six patients are alive; three enjoy excellent health, and none have developed evidence of progressive renal disease.

91. MEASUREMENTS OF THE TRANSFER OF AMNIOTIC FLUID INTO THE MATERNAL CIRCULATION DURING LABOR

JACK A. PRITCHARD, M.D., AND RICHARD A. SPARR, M.D. (BY INVITATION),
DALLAS, TEX.

Both the frequency and the consequences of amniotic fluid escaping into the maternal circulation during labor are debated. Immediate marked respiratory distress, hemorrhage due to abnormal hemostasis, or both have been associated with amniotic fluid embolization. Pulmonary vascular sclerosis has been suggested as a more remote result of a sublethal infusion of amniotic fluid. This study concerns attempts to detect any transfer of amniotic fluid into the maternal circulation during labor.

Because amniotic fluid did not produce appreciable hemolysis in vitro and because erythrocytes are similar in size to amniotic fluid squames, maternal erythrocytes from 50 ml. of blood were labeled with 250 μc of $Na_2Cr^{51}O_4$, washed, and injected transabdominally into the amniotic sac to "tag" the amniotic fluid. To rule out the possibility of transfer of radioactivity across an intact amniotic membrane, such erythrocytes were injected into the amniotic sac 24 hours prior to cesarean section in 6 women. No radioactivity appeared in the circulating maternal erythrocytes. The supernatant from centrifuged amniotic fluid contained no hemoglobin and less than 4 per cent of the total radioactivity.

In 12 women with intact membranes in whom labor was anticipated or was to be induced with intravenous pitocin, the amniotic fluid was similarly tagged with erythrocytes. The amniotic sac was ruptured during labor. Maternal blood was checked for radioactivity at the beginning of labor, at the time of rupture of membranes, at delivery, and the day following delivery. The maximum radioactivity in the circulating maternal erythrocytes was equivalent to the radioactivity in less than 10 ml. of amniotic fluid.

A small volume of amniotic fluid so "tagged" with erythrocytes and collected at the time of cesarean section was injected intravenously into a compatible subject with terminal carcinomatosis. The circulating erythrocyte radioactivity so produced agreed closely with the value calculated from the radioactivity injected and the subject's blood volume, suggesting that the infusion of any amniotic fluid into the maternal circulation during labor would have been detected with this technique.

It is concluded that during labor normally no more than trace amounts of amniotic fluid escape into the maternal circulation.

92. HYPERHEPARINEMIA—CLINICAL PICTURE AND TREATMENT

ARMAND J. QUICK, PH.D., M.D., AND CLARA V. HUSSEY, M.S. (BY INVITATION), MILWAUKEE, WIS.

The diagnosis of excess heparin in the blood which can be produced experimentally by the intravenous injection of this agent into normal subjects is determined by prolonged clotting, prothrombin, and thrombin times. Of particular diagnostic value is the normalization of the thrombin time by mixing plasma with a minute amount of protamine sulfate or toluidine blue before adding thrombin. A diagnosis of hyperheparinemia by means of these tests was made in a woman 23 years of age who has had a history of a bleeding condition since the age of 3 years, when she required six blood transfusions following a mastoidectomy. Bleeding severe enough to require transfusions has also occurred after tooth extraction and other minor operations. After the birth of her first baby, 34 transfusions were given before bleeding stopped, and after the second child, 28. Two months after the second parturition, the clotting time was α and the thrombin time 43 seconds (normal, 3 seconds). During the course of a year, the clotting time has remained 30 to 60 minutes and the thrombin time 7 to 55 seconds. The urgency for the removal of two adjacent molars and an impacted third molar prompted a study as to means for correcting the hyperheparinemia. The intravenous injection of protamine sulfate and of toluidine blue worsened the condition since the thrombin times increased. Following an oral dose of 100 mg. of cortisone, both the clotting and thrombin times decreased. Further reduction was obtained by giving additional cortisone and three 250 ml. units of fresh-frozen plasma. No abnormal bleeding occurred following the extraction of the three teeth. The patient's history suggests that since early childhood she has had a chronic hyperheparinemia sufficiently severe to keep the clotting time in the range of 30 to 60 minutes. Yet, she has bled severely on only a few occasions. She has not had internal bleeding, has always had normal menstruations, and only rarely has had abnormal bruises.

93. THE RELATIVE ABILITY OF RHEUMATIC AND NONRHEUMATIC CHILDREN TO RESPOND TO STREPTOCOCCAL ANTIGENS

ROBERT W. QUINN, M.D., NASHVILLE, TENN.

It has been shown that children who acquire rheumatic fever usually have higher streptococcal antibody titers than those who recover from streptococcal infections uncomplicated by rheumatic fever. Several investigations of the response of rheumatic and nonrheumatic persons to nonstreptococcal antigens

have shown no significant differences. A crucial question concerning the pathogenesis of rheumatic fever would seem to be: "Do children with rheumatic fever respond differently to streptococcal antigens than nonrheumatics?"

Streptolysin O concentrate was prepared and administered intramuscularly to 20 children with rheumatic fever and 55 nonrheumatic children. An additional group of 45 nonrheumatic children received no antigen.

The children were carefully observed clinically and determinations for antistreptolysin O, antistreptokinase, and antihyaluronidase performed before and 3 weeks after administration of the antigen.

The initial geometric mean antistreptolysin O titer of 158 combining units for the rheumatic children was higher than the initial mean titer of 121 for 100 nonrheumatic children; this difference is not statistically significant ($P=0.07$). Three weeks after the injection of antigen the mean level for the rheumatic children had risen to 221 units and for the nonrheumatics to 135, a statistically significant difference ($P=0.01$).

The initial mean antistreptokinase titer was higher for the rheumatic children but the mean antihyaluronidase titer was similar for all 3 groups studied. No significant rise in either of these mean antibody levels following antigen occurred, indicating that nonspecific or anamnestic antibody responses did not follow streptolysin O administration.

Six rheumatic children and 1 nonrheumatic had rather severe local and generalized reactions to the antigen. No evidence of initiation or reactivation of rheumatic fever was observed.

The results indicate that the group of children with rheumatic fever did have greater antistreptolysin O responses following the intramuscular injection of streptolysin O concentrate than did the nonrheumatic children. The significance of the findings is not clear but the results do support previous clinical observations that rheumatic children respond with higher antibody levels following a streptococcal infection than do nonrheumatic children.

94. THE NATURE AND MODE OF ACTION OF ERYTHROPOIETIC FACTOR IN ANEMIC PLASMA

W. A. Rambach, M.D. (By Invitation), J. A. D. Cooper, M.D., and H. L. Alt, M.D., Chicago, Ill.

Human and animal plasma factors capable of stimulating erythropoiesis in the laboratory animal have recently been demonstrated. The exact nature of these substances and their mode of action have not been defined. In the work to be reported the partial isolation and identification of an active principal from anemic plasma have been achieved through continuous flow paper electrophoresis, and ferrokinetic studies have been used to delineate its mode of action.

Boiled plasma extract obtained from phenylhydrazine-anemic rabbits has been found to contain several protein components when examined by paper electrophoresis. Separation by continuous flow electrophoresis yields an active fraction which migrates as an alpha-2 globulin. This material is heat stable and nondialyzable, and contains nitrogen and is not precipitated by perchloric acid. Paper electrophoretic patterns stain positively for carbohydrate and protein and do not accept a lipid stain. These findings strongly suggest the active erythropoietic principal to be a mucoprotein.

Albino rats have been injected subcutaneously with erythropoietic factor for seven days and then given an intracardiac injection of Fe^{59} as ferrous citrate. The uptake and disappearance of the radioiron were followed at 3, 6,

24, and 48 hours in the plasma, bone marrow, red blood cells, liver, and spleen and were compared with those observed in control animals injected with normal plasma or saline. In the treated animals plasma clearance is more rapid, there is a more rapid disappearance of the label from the bone marrow, the red cell label is higher, and there is increased uptake in the spleen and a decreased uptake by the liver. These findings in conjunction with elevated reticulocyte counts, hemoglobins, and hematocrits indicate an increased rate of cell division in, and an accelerated release of red cells from, the bone marrow of animals under the influence of erythropoietic factor.

95. THE SPECIFIC ADAPTATION SYNDROME

THERON G. RANDOLPH, M.D., CHICAGO, ILL.

The specific adaptation syndrome offers workable interpretations of hypersensitive, addictive, adaptive, "outgrowing," "hardening," and certain therapeutic clinical effects.

The specific adaptation syndrome resembles Selye's general adaptation except for greater degrees of specificity and sensitivity. Its successive nonadapted, adapted, and nonadapted stages, associated with increasing sensitization, probably involve similar endocrine and electrolyte mechanisms.

The test reaction (initial hypersensitiveness corresponding to the "alarm reaction") consists of immediate effects evoked either by cumulative or single specific doses. Although this nonadapted stage may be perpetuated by widely spaced doses, it may merge quickly with the following adapted state as a result of oft-repeated specific exposures.

The stage of *addiction* or apparent *hypo*sensitiveness is a prolonged specifically adapted state characterized by a *reversal of immediate effects.* Previous immediate postexposure symptoms taper off as general improvement occurs—provided regularly spaced, constant-sized to progressively larger and more frequent specific exposures are received. Because of the *pickup* after each dose and the subsequent timed *letdown* (withdrawal reaction), an amount to which one is quantitatively adapted is repeated often enough to enable the victim to ride the crests of the pickups, terminating or postponing his otherwise inevitable hangovers.

But such a "hair of the dog" treatment ultimately fails. Pickups decrease in degree and duration. Victims manifest an increasing level of localized and/or constitutional symptoms as well as regularly recurring predose hangovers—developments ordinarily reported as the "onset" of their present illnesses—as the nonadapted stage of exhaustion is approached.

That addiction (apparent hyposensitiveness and incorrectly called tolerance, desensitization, or immunity) is associated with high degrees of specific sensitivity may be readily demonstrated. Short-term avoidance is followed by initial withdrawal effects and subsequent reversion to the nonadapted test reaction stage in which a specific exposure induces immediate symptoms—the more prompt and severe, the greater the sensitization. But specific readdiction quickly occurs if oft-repeated exposures are resumed. Long-term avoidance leads gradually to reduced specific sensitivity.

Many are unknowingly addicted to foods, drugs, tobacco, chemical fumes, and other common materials. Although injectant routes are most efficient in inducing and maintaining specific addiction, inhalation, ingestion, and external contact are also effective.

96. CHANGE IN THYROID FUNCTION DURING STREPTOCOCCAL PNEUMONIA IN THE RAT AND ITS POSSIBLE SIGNIFICANCE FOR HOST RESISTANCE

SEYMOUR REICHLIN, M.D., PH.D. (BY INVITATION), AND ROBERT J. GLASER, M.D., ST. LOUIS, MO.

In order to study the effects of a severe bacterial infection on thyroid activity, rats were subjected to group A streptococcal pneumonia induced by a technique previously reported. Thyroid activity was determined by measurement of the rate of discharge of I^{131} from the gland and by changes in thyroid weight. Constant iodine intake was insured by parenteral administration of potassium iodide.

Decreased thyroid activity was noted in 9 of 14 infected rats, and began within 24 hours of inoculation in 8. The reduction was moderate to marked as compared to I^{131} discharge rates in control rats inoculated only with sterile mucin.

It was observed that the food intake of the infected animals was less than that of controls, and since starvation per se is associated with reduced thyroid activity, another experiment was done in which the thyroid activity of 12 infected animals was compared with that of pair-fed, noninfected controls. The results indicated that self-imposed food restriction apparently was the chief factor contributing to the thyroid inhibition which characterized pneumonic infections.

The thyroid glands of rats dying of pneumonia were significantly smaller than those of control animals whereas the adrenals were much larger. The thyroid inhibition occurring during the course of experimental pneumonia, however, was not secondary to increased adrenal cortical function since infected, adrenalectomized rats, maintained on constant parenteral doses of cortisone, showed thyroid inhibition comparable to that observed in infected, intact controls.

Finally, the possible influence of the level of thyroid activity on resistance to bacterial pneumonia was evaluated. Eighty rats were divided into three groups and all were fed 0.1 per cent propyl-thiouracil in their diets. Beginning three weeks prior to inoculation with streptococci, l-thyroxine was injected daily at three different dosage levels. It was found that hypothyroidism afforded some measure of protection as compared with the euthyroid or hyperthyroid state.

It is concluded that thyroid inhibition in experimental streptococcal pneumonia is primarily related to semistarvation, is not dependent on increased adrenal activity, and may well serve an adaptive function.

97. CHANGES IN BODY WATER COMPARTMENTS DURING THE COURSE OF ACUTE RENAL INSUFFICIENCY

ALEXANDER P. REMENCHIK, PH.D. (BY INVITATION), JAMES A. SCHOENBERGER, M.D., AND JOSEPHINE M. DYNIEWICZ (BY INVITATION), CHICAGO, ILL.

Although changes in body water compartments in acute renal insufficiency have been extensively studied in experimental animals, only a limited number of observations of man have been published. From one to three simultaneous studies of antipyrine space (AS) and radiosulfate space (RSS) were made in eight patients with acute renal insufficiency. All patients, after diagnosis, were managed during the oliguric phase by continuous intravenous admin-

istration of hypertonic dextrose in water in amounts less than 600 ml./24 hours. In six of the patients the initial AS was greater than 60 per cent of the body weight. In three patients, who recovered from acute renal insufficiency, there was a marked fall in AS to values within normal limits. In one patient studied frequently during the period of oliguria, the initial AS was in the normal range and subsequent changes in AS correlated with changes in body weight. The remaining four patients expired before additional studies were done.

The initial RSS was greater than 20 per cent in all of the patients. In the three patients who survived, the RSS fell to normal in two patients and to near normal in the other patient. In contrast to other types of patients, there was no significant loss of radiosulfate from the RSS into sinks other than the urine during the oliguric phase.

The results are in agreement with the conclusions of other investigators that there is an accelerated net catabolic rate in acute renal insufficiency. This increased catabolism of body tissue produces a quantity of water larger than had been previously realized, and the diuretic phase in part represents the excretion of excess quantities of water and electrolytes accumulated during the catabolism of this tissue. If this interpretation is correct, then the therapy of acute renal failure should be modified accordingly.

98. MECHANISMS AND CLINICAL SIGNIFICANCE OF THE AUSCULTATORY GAPS IN AORTIC STENOSIS, HYPERTENSION, AND ARTERIOSCLEROTIC HEART DISEASE

SIMON RODBARD, M.D., PH.D., BUFFALO, N. Y.

The frequent occurrence of auscultatory gaps in aortic stenosis and in systolic hypertensions suggests an association of this sign with a general cardiovascular disturbance. The patterns associated with the gap were studied in more than 100 patients in whom it was present spontaneously, or in whom it could be induced. The gap was constantly associated with the level of a shoulder (incisura) on the upstroke of the arterial pulse wave. This relationship between incisura and gap was demonstrated by direct brachial artery puncture, and by indirect construction of the arterial pulse wave, using arteriophonography. The wider the gap, the greater the halt on the upstroke was found to be. In hypertension and in arteriosclerosis with high systolic pressures (80 cases) the gap was usually at 170 mm. Hg. In aortic stenosis (20 cases) it was at a level of about 110 mm. Hg.

The auscultatory gap could be eliminated in every instance by increasing the blood flow to the arm, as by reactive hyperemia. The incisura was unaffected by this maneuver. Reduction in the blood flow to the extremity by application of a tourniquet below the stethoscope bell widened the gap; this maneuver also elicited gaps in patients with incisura on the upstroke of the pulse wave who also had reduced cardiac reserves. It did not elicit gaps in normal subjects.

The diminution in the amplitude of the sound is associated with the arterial "opening snap" generated when the rising arterial wave equals and then exceeds the pressure in the sphygmomanometer cuff. A reduced slope at an incisura produces a weaker snap, and this may be inaudible. The second phase of the arterial sounds, which has a murmurlike quality and is dependent on the volume of blood flowing into the extremity, provides a measure of the vascular resistance in the extremities, which is apparently elevated in the conditions mentioned.

This analysis suggests that in conditions associated with a reduced cardiac reserve, the blood flow to the extremities is restricted and the arterial sounds are fainter. The presence of a gap provides useful data concerning cardiovascular function.

99. THE DISC METHOD TUBERCULIN TEST

(A Simple, Sensitive, One-Dose Test)

Sol Roy Rosenthal, M.D., Ph.D., and Elvyra H. Katele, M.D.
(By Invitation), Chicago, Ill.

The pitfalls in tuberculin testing by the Mantoux method are many. Leaking syringes, injecting too deeply, and difficulty in interpretation of the test lead to many discrepancies. Recently, the Laboratory of Biologics Control has recommended that a new syringe and needle be used for each tuberculin injection by the Mantoux method in order to minimize the possibility of transmitting infectious hepatitis.

The tuberculin test here reported uses a stainless steel disc $\frac{5}{16}'' \times \frac{7}{16}''$ that has 4 needles or prongs. The holder is a small magnet. This is a modification of the multiple puncture disc method of BCG vaccination described by the senior author in 1948. The method of application is to place a drop of concentrated O.T. on the arm and puncture through the drop. Readings for children are best made after 96 hours, and for adults after 48 hours. A positive reaction consists of papules more than 1 mm. in diameter. The method in practically painless and foolproof. The interpretations are simple. The report will be based on some 800 children and young adults. As for the incidence of false negative reactions, in one group of children (6 to 14 years) there was 1 of 65 who were negative to the disc test that was positive to 1:1,000 of O.T. by the Mantoux method. In one group of young adults (17 to 24 years), there were 3 of 35 negative to the disc test and positive to 1:1,000 of O.T. As for false positives there were 2 children of 105 who were positive to the disc test and negative to 1:100 O.T. There were no false positives among the adults. The sensitivity of the test lies between 1:1,000 and 1:100 O.T. by the Mantoux method. This test would lend itself for use by the private physician especially since no dilutions or needle and syringe are necessary. The concentrated tuberculin is contained in single-dose capillary glass tubes. The test is likewise suitable for epidemiological studies because of its ease of administration and interpretation.

100. RESPONSE OF PULMONARY ARTERY PRESSURE AND TOTAL PULMONARY RESISTANCE OF NORMAL MAN TO PROLONGED MILD, STEADY-STATE EXERCISE

Salvatore M. Sancetta, M.D., and Louis Rakita, M. D. (By Invitation),
Cleveland, Ohio

The effect of a 39-minute period of exercise (850 foot pounds/minute, steady state having usually been achieved in 9 minutes) on the pulmonary hemodynamics has been investigated in 12 normal and untrained subjects in the supine position, employing a bicycle ergometer. Pulmonary artery pressures (PPA) were recorded every minute during a 5-minute control period, during a 5-minute period of stationary, leg-up position, and during a 39-minute period of exercise, except when duplicate cardiac outputs (Fick) were being determined (control, 10 to 14, 20 to 24, 30 to 34 minutes of exercise).

PPA increased in all cases but one after the legs were raised in position but kept stationary. This is thought to be due to displacement of blood into the lungs. Exercise produced a rapid increase in PPA which began to decrease before the end of 9 minutes and showed a progressive decrease throughout the remainder of exercise. Total pulmonary resistance (Pu.T.R.) decreased progressively throughout the entire experiment. *These progressive decreases in PPA and Pu.T.R. were independent of changes in total ventilation, respiratory rate, and cardiac output.* Similar PPA decreases occurred in 3 subjects in whom no blood samples were withdrawn; therefore this decrease is not due to blood loss in sampling.

In 3 patients the exercise load was doubled at the 30-minute period of exercise, the latter being continued another 20 minutes. There was a secondary initial rapid rise in PPA above the pressure recorded at the 30-minute period with a secondary decrease below the 30-minute level by the end of the experiment. Pu.T.R. showed a further decrease with the increased load.

There was no relation between PPA and brachial artery pressure changes, although total peripheral resistance progressively decreased.

The decreases in PPA and Pu.T.R. are thought to be due to progressive relaxation of the pulmonary vascular bed, triggered by an increased but later unchanging venous return, or the progressive opening of pulmonary capillaries and/or precapillaries not functional in the basal resting state, or to a combination of both situations.

101. EXPERIMENTAL PRODUCTION OF ANHIDROSIS AND HYPOHIDROSIS BY DIET AND DEHYDRATION

FREDERICK SARGENT, II, M.D., ROBERT E. JOHNSON, M.D., RICHARD A. HUNTLEY, M.S., RICHARD KOSMALA, B.S., AND HENRY H. HANLEY, URBANA, ILL.

(INTRODUCED BY ROBERT H. EBERT, M.D.)

Whether dehydration will significantly reduce rate of thermal sweating in man has not been definitely established. This problem was studied during a 36-day field test in two ways: (1) by clinical observation and (2) by means of a standard test of heat tolerance. For nine days, 88 healthy young men in groups of 2 to 4 subsisted on controlled regimens of unlimited and limited (910 ml./day) water intake; low osmotic load (pure carbohydrate); high osmotic load (high protein-low salt, or moderate protein-high salt); or intermediate osmotic load (medium protein or salt). Half marched 12 miles/day; half 3 miles/day. In a 14-day prerecovery period and 13-day recovery period they subsisted on a standard packaged ration and unlimited water, and engaged in moderate work. Twelve controls subsisted throughout on unrestricted amounts of fresh foods; water was unrestricted; work load was moderate. Mean maximum temperatures were 90° F. in pre-, and 94° in experimental and recovery periods. Three subjects on restricted water developed total anhidrosis; eight others clinical hypohidrosis. Body temperatures were only slightly elevated when sweating deficiency was first detected. There was no miliaria. Hyperpyrexia did not develop because the men were promptly treated with oral fluids. When water ingestion began, a number exhibited, within 5 minutes, an outburst of sweating. All the subjects were in negative water balance. Water intake of 2,700 ml./day prevented further occurrence of anhidrosis. Five tests of heat tolerance were made during which subjects in groups of 25 marched one hour at 3.75 m.p.h. under outdoor weather (effective temperature, 65.1° to 82.4°). Observations were made of rectal and skin temperature and pulse rate. In the experimental period the 88 subjects exhibited signifi-

cant reduction in rate of sweating. Only those on limited water became heat intolerant as judged by Ladell's acclimatization index (sweat rate/increment of rectal temperature); men on high osmotic loads were most heat intolerant. We speculate that we were dealing with several degrees of sweat gland fatigue precipitated by dehydration.

102. CLINICAL AND LABORATORY STUDIES IN AN INTRAFAMILIAL EPIDEMIC OF PHARYNGOCONJUNCTIVAL FEVER

SAMUEL SASLAW, M.D., PH.D., ROBERT G. VAN HORNE, M.D. (BY INVITATION), GEORGE R. ANDERSON, D.V.M. (BY INVITATION), FREDERICK J. FLATLEY, M.D. (BY INVITATION), AND RICHARD D. CARR, M.D. (BY INVITATION), COLUMBUS, OHIO

A recent intrafamilial epidemic of pharyngoconjunctival fever offered us an opportunity to study both the clinical and laboratory aspects of the disease. Clinical findings consistent with pharyngoconjunctival fever were associated with successful Type 3 virus isolations, and significant elevations of specific serologic titers in three siblings. No virus was recovered from a fourth sibling, who was recovered from his disease when first seen, but serologic evidence of disease was obtained. A fifth sibling and both parents were unaffected. The clinical symptoms in the affected children were strikingly similar in that all exhibited fever, conjunctivitis, pharyngitis, rhinitis, malaise, and cervical adenitis. Transient diarrhea was present in two of the four children. All responded to supportive therapy alone. The incubation period was apparently between five to eight days as the disease progressed through the family.

Group-specific complement-fixing antibodies were absent after seven months, although type-specific neutralization antibodies were still present in similar titers to those observed one month after onset. This dissociation of serologic tests suggests that the complement-fixing titer is relatively transient in nature.

Correlated clinical-laboratory studies in relatively new recognized disease syndromes, once applied thoroughly in early experiences, facilitate the clinical diagnosis in subsequent patients, and decrease the need for extensive laboratory studies once that experience has been firmly established.

103. PREFERENTIAL BINDING AND ABSORPTION OF CYANOCOBALAMIN IN THE PRESENCE OF EXCESS PSEUDOVITAMIN B_{12}

ROBERT F. SCHILLING, M.D., MARY BARTLETT BUNGE, M.S. (BY INVITATION), AND LEE L. SCHLOESSER, M.D. (BY INVITATION), MADISON, WIS.

When the benziminazole moiety of cyanocobalamin is replaced by adenine the resulting compound, lacking in antipernicious anemia activity, is known as pseudo-vitamin B_{12}. Gastric juice binds cyanocobalamin in a nondialyzable bond up to a certain maximum quantity of cyanocobalamin per unit of gastric juice. Pseudo-vitamin B_{12} offers little if any competition to cyanocobalamin for the cyanocobalamin binding sites in normal human gastric juice. In human serum, there is a strong competition between cyanocobalamin and pseudovitamin B_{12} for the sites to which cyanocobalamin can be bound. These facts are interpreted as evidence that there is a fundamental chemical difference between the gastric juice–cyanocobalamin bond and the serum–cyanocobalamin bond.

If a patient who lacks intrinsic factor is given gastric juice plus a mixture of 1 μg of radioactive cyanocobalamin and 50 μg of nonradioactive cyanocobalamin he will absorb very little of the radioactive vitamin because of the isotope dilution effect on the small total quantity of vitamin B_{12} absorbed. If, a few days later, the same subject is given gastric juice plus a mixture of 1 μg of radioactive cyanocobalamin and 50 μg of nonradioactive pseudo-vitamin B_{12} he will absorb the radioactive vitamin "normally," i.e., just as if he were given the radiovitamin and gastric juice with no other form of vitamin B_{12}. These data are evidence that pseudo-vitamin B_{12} is not in competition with cyanocobalamin for the physiologic mechanisms effecting the absorption of cyanocobalamin.

The effects of other analogues of vitamin B_{12} as competitors with cyanocobalamin in these in vitro and in vivo systems will be presented.

It is believed that the correlation between selective in vitro binding of cyanocobalamin by gastric juice and the selective in vivo absorption of cyanocobalamin in the midst of an excess of pseudo-vitamin B_{12} is evidence that a unique type of cyanocobalamin binding is an essential property of intrinsic factor.

104. THE CONJUGATION OF BILIRUBIN WITH GLUCURONIC ACID

RUDI SCHMID, M.D., BETHESDA, MD.

It has long been known that in jaundiced serum, bilirubin occurs in forms of a "direct" and an "indirect" reacting pigment, but the factors responsible for this difference have not been fully understood. Recently, several investigators working with semipurified protein-free pigment fractions have presented evidence which suggested a marked difference in solubility between these two types of bilirubin.

It has now been found that the "direct-reacting" water-soluble bilirubin in jaundiced serum and urine, and in normal bile, is bilirubin glucuronide, whereas the "indirect-reacting" bilirubin of jaundiced serum is free bilirubin. These results were obtained by studying the stable dipyrromethene diazonium pigments which resulted from treatment of biologic fluids containing bilirubin with an excess of diazotized sulfanilic acid. After preliminary purification, the azopigments were separated by paper chromatography. It was observed that "direct-reacting" bilirubin of serum, bile, and urine resulted in azopigments, which were conjugated with glucuronic acid. By heating the conjugated azopigments in hydrochloric acid or by incubating them with β-glucuronidase, free glucuronic acid and free azopigment were obtained in a 1:1 molar ratio. The latter compound was found to be identical with the azopigments, resulting from diazotization of "indirect-reacting" bilirubin and crystalline bilirubin.

In human bile, most of the bilirubin is excreted as glucuronide. In obstructive and hepatogenous (regurgitation) jaundice, glucuronic acid-conjugated bilirubin gains access to the blood, where it is detectable as "direct-reacting" bilirubin. Electrophoretically both bilirubin fractions, the free ("indirect") and the conjugated ("direct") migrate with the serum albumin. In jaundiced urine, only conjugated bilirubin could be detected.

In patients with congenital, *nonhemolytic* jaundice, the main defect appears to consist of a greatly reduced ability of the liver to conjugate bilirubin and other compounds with glucuronic acid. In hepatectomized dogs, the "golden pigment," which accumulates in the plasma and is excreted in the urine, seems to be related to bilirubin but it does not appear to be a glucuronide of bilirubin.

105. A CORRELATIVE STUDY OF A VASOMOTOR AND A GALVANIC SKIN REFLEX AS INDICATORS OF CENTRAL SYMPATHETIC REACTIVITY

ROBERT A. SCHNEIDER, M.D., AND J. PAUL COSTILOE, M.S. (BY INVITATION),
OKLAHOMA CITY, OKLA.

The authors have previously demonstrated that certain C.N.S. depressant agents inhibit the conditioned galvanic skin response and also depress central sympathetic reactivity as measured by the Funkenstein test where the blood pressure response to the hypotension resulting from injection of 10 mg. Mecholyl is monitored for a 25-minute period. Gellhorn has shown that the mechanism responsible for the recovery of blood pressure is a reflex sympathetico-adrenal response, the magnitude of which depends on the subject's central sympathetic reactivity. Thus, subjects can be classified as having normal, hyper- or hypo-reactivity. Furthermore, there is evidence that the amplitude of the conditioned galvanic skin response and the central sympathetic reactivity is greater in anxious than in nonanxious subjects. Thus, it appears that the amplitude of the conditioned response is related to the subject's central sympathetic reactivity. The present study tests the hypothesis that subjects who are classified as having high central sympathetic reactivity by the Funkenstein test will show a greater increase in galvanic skin response amplitude in a conditioning situation than subjects with low central sympathetic reactivity.

The galvanic skin response was conditioned and extinguished in twenty subjects using a classical conditioning procedure with a light as the conditioned and an electric shock as the unconditioned stimulus. Ten minutes later each subject was given the Funkenstein test. Data from nine of the subjects were analyzed—three who were clear-cut hyper-reactors to Mecholyl, three normal reactors, and three obvious hyporeactors. Borderline reactors were excluded.

RESULTS

Our hypothesis was supported. (1) The amplitude of the galvanic skin response was greatest in those subjects showing central sympathetic hyper-reactivity on the Funkenstein test; (2) it was only meager in those subjects whose Funkenstein test showed hyporeactivity; and (3) it was midway in amplitude in subjects classified as normal reactors by the Funkenstein test. Correlation coefficients between these two measures were significant ($P < 0.01$). These positive correlations between two dissimilar sympathetic reflexes suggest that the changes in galvanic skin response amplitude to meaningful stimuli may also give an estimate of central sympathetic reactivity.

106. THE INDUCTION OF MOUSE LEUKEMIA BY CELL-FREE FILTRATES OF LEUKEMIC MOUSE AND LEUKEMIC HUMAN BRAIN

STEVEN O. SCHWARTZ, M.D., HAROLD M. SCHOOLMAN, M.D. (BY INVITATION),
PAUL B. SZANTO, M.D. (BY INVITATION), AND WILMA SPURRIER
(BY INVITATION), CHICAGO, ILL.

Cell-free filtrates were prepared from the tumor and brain of a white Swiss mouse which developed a "spontaneous" lymphoblastoma. The filtrates were passed through a Seitz sterilizing pad whose integrity was tested by a saline suspension of *Escherichia coli.*

Thirty-five of sixty white Swiss mice inoculated intracerebrally, and thirty-five of forty-five mice inoculated intraperitoneally with the brain filtrates developed leukemia. The mice were five to eight weeks old at the time of inoculation. Ninety per cent of the animals which developed leukemia did so within 25 days following inoculation.

Control animals received inoculations of heat-inactivated (65° C. for 45 minutes) filtrates of leukemic brain. One mouse of 49 injected intracerebrally, and none of 10 inoculated intraperitoneally developed leukemia.

None of the 45 mice inoculated intracerebrally or the 44 mice inoculated intraperitoneally with filtrates of tumor developed leukemia.

Cell-free filtrates were also prepared from the brains of patients dying of leukemia. These were inoculated into adult AKR mice. Leukemia developed in 63 of 306 mice before the animals reached the age of 22 weeks. Regardless of their age at the time of inoculation, the age of 22 weeks was chosen to terminate the experiments because this is the age at which spontaneous leukemia begins to occur in our stock. The appearance of leukemia in the inoculated animals before the age of 22 weeks, therefore, could be interpreted as related to the inoculation. None of 208 animals injected with various control filtrates developed leukemia before the age of 22 weeks.

107. PRIMARY PULMONARY EMPHYSEMA IN MIDDLE-AGED ADULTS

PAUL M. SEEBOHM, M.D., GEORGE N. BEDELL, M.D. (BY INVITATION), AND JUNE M. FISHER, M.D. (BY INVITATION), IOWA CITY, IOWA

Severe pulmonary emphysema developing in early middle life without evidence of other significant lung disease to account for its causation has been defined as primary in this paper. Although the existence of this condition has been acknowledged, it has been inadequately described and is still poorly understood. The purpose of this paper is to present the clinical and laboratory findings in 10 patients with primary pulmonary emphysema. The cough and wheeze present in some of the patients was considered too mild to be the cause of the severe incapacitating emphysema seen at an early age in this series.

The 10 patients described here varied in age from 38 to 55 years. The age of the patients at onset of symptoms referable to emphysema was 14 to 52 years. All patients complained of shortness of breath on exertion which became progressively worse. Coughing was present in 5 patients. Wheezing was present in 5. Coughing and wheezing were absent in 4 patients. Physical examination revealed the classical findings of emphysema of which the most striking was diminished breath sounds over the bases regardless of chest configuration. This finding occurred in every patient. Chest x-rays revealed increased aeration of the lungs and depressed diaphragms without evidence of parenchymal infiltration. Inhalant allergy skin tests performed in 6 patients were negative.

Pulmonary function tests showed that the vital capacity varied from 22 to 85 per cent of predicted value; residual volume from 162 to 524 per cent of predicted value (9 patients); single-breath nitrogen test 9 to 12 per cent (normal less than 1.5 per cent) (5 patients); per cent nitrogen after 7 minutes of oxygen breathing varied from 2.5 to 18 per cent; maximal breathing capacity 19 to 36 per cent of predicted; maximal expiratory flow rate 15 to 66 L./min. (normal 400-600 L./min.) (8 patients). Arterial oxygen saturation at rest breathing room air varied from 51 to 91 per cent (9 patients), and arterial P_{CO_2} tension 47 to 61 mm. Hg (7 patients).

108. NONPRECIPITATING INSULIN-BINDING ANTIBODIES

JOSEPH H. SKOM, M.S., M.D. (BY INVITATION), AND DAVID W. TALMAGE, M.D., CHICAGO, ILL.

The existence of antibodies to insulin is well established, but their relationship to the pathogenesis of diabetes and to insulin allergy remains obscure. The quantitation of insulin binding by human globulins has been accomplished by a method that is sensitive, precise, and sufficiently practical to be used as a routine clinical determination. Two-thousandths microgram of I^{131} insulin in 0.5 ml. of buffered saline is incubated for one hour at 4° C. with 0.5 ml. of 1:25 human serum. Rabbit antihuman globulin serum (AHG) is added in an amount which produces maximum precipitation of the human globulins. After standing overnight at 4° C. the precipitate is centrifuged, washed once with cold saline, and counted. The quantity of insulin bound can be calculated by comparing the number of counts in the precipitate with the number of counts in exactly 0.002 μg of the same I^{131} insulin set up as a standard. The avidity of the antibody to insulin may be determined by a comparison of insulin binding at two different concentrations of insulin. Electrophoretic studies of AHG alone and of sera before and after precipitation with AHG have demonstrated that all insulin-binding antibodies are precipitated by AHG and that AHG itself does not bind insulin. This method is now being used routinely to determine the insulin-binding capacity of the serum of diabetic patients. In preliminary results, insulin-binding antibodies have been found in all insulin-treated subjects and only in insulin-treated subjects. Serial studies begun on diabetic patients prior to treatment with insulin should provide information concerning the characteristics of the antibody response. The relationship of these antibodies to human insulin is being studied by the same methods. The use of this system to assay insulin is being investigated and the application to other endocrine systems seems likely.

109. THE INCORPORATION OF C^{14} FORMATE INTO THE PROTEIN FRACTIONS OF NORMAL AND METASTATIC HUMAN CANCER

HOWARD H. SKY-PECK, PH.D. (BY INVITATION), SYDNEY KOFMAN, M.D. (BY INVITATION), DEVANABOGINA NAGAMANI, M.D. (BY INVITATION), RICHARD J. WINZLER, PH.D. (BY INVITATION), AND SAMUEL G. TAYLOR, III, M.D., CHICAGO, ILL.

In order to more objectively evaluate the clinical effectiveness of chemotherapy and ablative procedures in the treatment of disseminated human breast cancer, an in vitro comparison has been attempted between the metabolic rates of normal and neoplastic mammary tissues during the course of therapy. Biopsy material was taken from normal mammary tissue and neoplastic breast tissue before, during, and after therapy. These tissues were sliced and alternate slices fixed for pathologic evaluation while other slices were incubated at 38° C. in a Krebs-Ringer medium containing an excess of radioactive formate and nonlabeled glucose. At intervals of 2.5 and 5.0 hours incubations were terminated and the tissues fractionated into their acid-soluble, lipid, protein, and nucleic acid components. The radioactivity incorporated into the protein fractions was determined and expressed as relative specific activity. The ribonucleic acid and desoxyribonucleic acid present in these tissues were quantitatively estimated in an attempt to evaluate the relative amounts of tumor cells compared to normal cells present in any given section of tissue.

Preliminary results indicate that the rate of incorporation of C^{14} from formate into the normal tissue proteins remains relatively constant for any given individual during the course of therapy; whereas, the rate of C^{14} incorporated into the neoplastic tissue proteins increased or decreased with the clinical progression or regression of the disease. Two patients with very rapidly growing neoplasms showed an unusually high rate of incorporation of C^{14} into their tumor proteins as compared with patients having slow growing tumors. Two patients previously showing progression of disease had clinical improvement following oophorectomy with a concomitant decrease in the rate of C^{14} uptake into the tumor tissues. In another patient a depression was also detected in the rate of C^{14} uptake into the tumor proteins within seven days after the institution of testosterone therapy. However, regression was not clinically demonstrable for several weeks. Two patients biopsied before and seven days after the administration of stilbestrol showed a stimulation of C^{14} uptake. Work is still in progress on a larger number of patients with metastatic breast cancer.

109a. STAPHYLOCOCCUS ALBUS SEPTICEMIA

IAN MACLEAN SMITH, M.D., AND PHYLLIS BEALS, IOWA CITY, IOWA

(INTRODUCED BY RAYMOND F. SHEETS, M.D.)

The occurrence recently of *Staphylococcus albus* septicemia in two post-valvulotomy patients caused us to re-examine this subject. At our hospital in a 20-year period during which 410 cases of *Staphylococcus aureus* septicemia occurred there were also eight cases of septicemia due to the albus variety of the Staphylococcus. No doubt this number should be greater but certain cases had not been adequately investigated to establish the diagnosis beyond doubt. These cases along with more than 30 others found in the literature have been studied from a clinical point of view. The disease occurs particularly during periods of reduced host resistance such as in the neonatal, post-partum, and postoperative periods. It often affects patients with hearts damaged congenitally or by rheumatic fever. The joints and pericardium are frequently affected. The disease is commonly fatal.

The organisms from three such cases have been studied in detail and compared with *Staphylococcus aureus* strains isolated from various lesions. The survival of the *Staphylococcus albus* strains in the tissues of Swiss albino mice has been followed. Attempts have been made to alter host resistance in these mice by starvation, by starvation plus oral glucose, by cardiac trauma, by cortisone, and by other methods thought to simulate the conditions found in the naturally occurring disease of man.

110. FAILURE OF SPECIFIC ANTIBODY PRODUCTION IN RABBITS INDUCED BY NEONATAL EXPOSURE TO ANTIGEN

RICHARD T. SMITH, M.D., ROBERT A. BRIDGES, M.D., AND ENID THOMSON, MINNEAPOLIS, MINN.

(INTRODUCED BY CHANDLER A. STETSON, M.D.)

Litters of rabbits were given various amounts of crystalline bovine albumin (BSA) intraperitoneally on the first or designated day of life. At varying intervals attempts were made to induce antibody formation in groups of animals so exposed to BSA and other protein antigens. Rabbits given quantities of BSA exceeding 10 mg. before 12 hours of age were unable at 4 and 8 months of age to produce any precipitating antibody. Further, antigen injected at 4

and 8 months was catabolized at a rate identical to endogenous albumin. Fifty per cent and 30 per cent of groups receiving 10 mg. and 1 mg., respectively, failed to respond to subsequent antigenic challenge. Animals receiving 0.1 mg. or less were found to have the same incidence of nonresponders as control groups (3-9 per cent).

Failure of subsequent response was shown in all groups of animals receiving 100 mg. BSA on the first or third day of life, and in 50 per cent and 30 per cent, respectively, of those getting the neonatal dose on the ninth or fifteenth day of life. Rabbits in the fifteen-day group which produced antibody showed a secondary-type immune response.

The high degree of specificity of this unresponsive state was shown by normal antibody production in response to other protein antigens.

Neonatal exposure by several routes to large amounts of bacterial antigen in all experiments thus far has failed to prevent: (1) subsequent specific antibody formation, (2) development of bacterial hypersensitivity, (3) alteration of response to specific infection, or (4) reactivity to bacterial endotoxin.

The results indicate that exposure to threshold amounts of heterologous protein shortly after birth in rabbits results in failure of antibody production following any subsequent specific antigenic challenge. This alteration carries with it a degree of obverse specificity equivalent to that of the immune response itself. By the fifteenth day most infant rabbits respond in a manner which allows a normal subsequent specific immune response with antibody production.

These experiments are currently being extended to other mammalian species, and a variety of other antigens.

111. EFFECTS OF PHENYLBUTAZONE ON SERUM CONCENTRATION AND RENAL CLEARANCE OF URATES: A LONG-TERM STUDY IN GOUTY AND NONGOUTY ARTHRITICS

CHARLEY J. SMYTH, M.D., GEORGE M. WILSON, JR., M.D. (BY INVITATION), AND ELSTON R. HUFFMAN, M.D. (BY INVITATION),
DENVER, COLO.

Previous studies have demonstrated a decrease of 40 per cent in the serum urate concentration in both gouty and nongouty patients following phenylbutazone administration. This change is due to an increase in the renal clearance of urate. The present study was undertaken to determine whether or not the lowering of the serum urate level attending phenylbutazone ingestion could be sustained for long periods. Four patients with gout and four nongouty arthritics were maintained on 200 to 400 mg. daily oral doses of phenylbutazone for 59 to 420 days. Observations on the renal clearance of inulin, para-aminohippuric acid, sodium, potassium chlorides and urates were made before and at one- to three-month intervals. Standard renal clearance techniques were used utilizing a constant infusion pump and bladder washouts.

The effect of long-term phenylbutazone administration on the clearance ration C_{Ur}/C_{In} in the gouty subjects was an increase from a mean of 7.4 to 12.4 (67.6 per cent). The nongouty subjects had an increase in the C_{Ur}/C_{In} from a mean of 10.0 to 18.9 (87.0 per cent).

The concentration of the serum urates in the gouty subjects decreased from a mean control value of 8.2 to 6.7 mg. per cent (18.2 per cent) while the nongouty arthritics decreased from a mean control value of 5.1 to 3.6 mg. per cent (29.4 per cent) following administration of the drug. Phenylbutazone levels in excess of 10 mg. per cent were necessary to maintain a sustained reduction in the serum urates. Two subjects received probenecid in combina-

tion with phenylbutazone and in both there was a further decrease in the serum urate concentration as compared with that obtained with phenylbutazone alone.

There was no constant change in the clearance of sodium, potassium, or chloride. There was a slight increase in the filtration fraction due to a decrease in the C_{PAH} in 6 of the 8 subjects studied.

None of the 4 patients with gout (treated for 176, 59, 361, and 263 days, respectively) had an acute attack of gout during the period of study and no toxic reactions occurred.

112. SERUM CHOLESTEROL AND CHOLINESTERASE FOLLOWING INFUSION OF HUMAN SERUM ALBUMIN IN HEALTHY SUBJECTS AND PATIENTS WITH THE NEPHROTIC SYNDROME

JOHN F. SOOTHILL, M.B., M.R.C.P., CHICAGO, ILL.

(INTRODUCED BY DOUGLAS A. MACFADYEN, M.D.)

A reciprocal relationship exists between the serum levels of cholesterol and cholinesterase and that of albumin in the nephrotic syndrome. All three substances are synthesized in the liver. The synthesis of albumin is probably increased in nephrotic syndrome because of urinary loss. The serum levels and the rates of synthesis of all these three substances are decreased in hepatocellular dysfunction and in malnutrition. It is suggested that their rates of synthesis might be interrelated and dependent on the serum albumin level. We report results of an experiment designed to study this relationship.

Human serum albumin, 50 Gm. per day, was infused for either nine or eighteen days into three healthy subjects and into three patients with nephrotic syndrome. The subjects were living on fixed regimens in a metabolic ward during the period of infusion, for six days before, and nine days after. Throughout this period studies were made of the serum cholesterol, cholinesterase, total protein with electrophoretic partitions, electrophoretically separated alpha and beta lipoprotein cholesterol, hematocrit, body weight, and urinary protein. In one patient albumin space, total lipids, and phospholipids were measured.

In the healthy subjects the serum cholesterol fell from the order of 200 mg. to 120 mg./100 ml. In the nephrotics, the level fell by 50 per cent. The 40 to 50 per cent fall in serum cholinesterase, previously reported in healthy subjects following albumin infusions, was confirmed. A similar fall was demonstrated to occur in nephrotics. In all cases the fall in the serum level was too great to be accounted for by the increase in plasma volume, and it coincided with the rise in serum albumin level.

The mechanism by which these changes occur will be discussed in terms of hepatic synthesis and serum transport of cholesterol.

113. THE INFLUENCE OF PITOCIN ON RENAL FUNCTION IN PATIENTS WITH PITUITARY INSUFFFICIENCY, RENAL DISEASE, AND HYPERTENSION, AND IN NORMAL PATIENTS

C. L. SPURR, M.D., AND R. V. FORD, M.D. (BY INVITATION), HOUSTON, TEX.

Pitocin will restore reduced hemodynamic relationships in hypophysectomized dogs (Demunbrun and co-workers). The effect of this hormone in human patients presenting pituitary insufficiency has been studied.

For comparative purposes, the study has been extended in precisely the same fashion to include equal numbers (three) of patients in five groups: (1) pan-hypopituitary patients; (2) renal disease; (3) hypertensive disease; (4) patients receiving Meticorten for rheumatoid arthritis; and (5) normal patients. Each of these five categories was comprised of three patients.

Renal functions were studied in the control state and following the seven consecutive days' administration of Pitocin as 1 ml. Q 4 H, subcutaneous injection. The studies consisted of the measurement of the clearance of inulin (GFR), clearance of PAHA (RPF), tubular excretory capacity (Tm_{PAH}), the excretion rate of sodium, potassium, and water, and the various ratios resulting from these determinations.

In none of the five categories was there a significant change in glomerular filtration rate, renal plasma flow, tubular excretory capacity, or the excretion rate of water, sodium, or potassium. There was no change in antidiuretic effect as determined by the inulin U/P ratio or in blood pressure. In patients with hypopituitarism and in patients with glomerulonephritis, there was no change in renal hemodynamics (GFR and RPF), but there was a significant increase in the tubular excretory capacity for PAHA. The increase in the hypopituitary patients' control Tm_{PAH} was from 64 to 92 mg. per minute (42 per cent), $p < 0.001$. In the group with renal disease, the increase was from 83 to 98 mg. per minute (18 per cent), $p < 0.001$. Other groups show less than 5 per cent variation. This single-dose Pitocin effect could not be demonstrated in acute eight-hour studies. The data confirm the work in hypophysectomized dogs concerning the restoration toward normal of renal function. The data add the fact of an increased renal function (Tm_{PAH}) in the patient with glomerulonephritis after the administration of Pitocin.

In view of the absence of this effect in other patients having normal renal function, it is suggestive of a pituitary deficiency in the patient with glomerulonephritis.

114. A METHOD FOR STUDYING HEPATIC BLOOD FLOW USING RADIOACTIVE IODINATED SERUM ALBUMIN AND EXTERNAL COUNTING TECHNIQUES

JOHN P. STORAASLI, M.D., RICHARD GORLIN, M.D. (BY INVITATION), STANLEY REICHMAN, M.D. (BY INVITATION), AND WILLIAM D. DAVIS, JR., M.D.,
PORTSMOUTH, VA.

Utilizing a scintillation probe counter placed externally over the liver, and continuous recording equipment, a curve of radioactivity is obtained which reflects the manner in which iodinated serum human albumin is diluted in the hepatic vascular bed following rapid intrasplenic injection. Fifty patients have been studied by this technique. These included patients with no liver disease, those with acute and convalescent hepatitis, and those with cirrhosis, with or without esophageal varices. In fifteen of the patients simultaneous estimations of hepatic blood flow were made using the multiple blood sampling technique, the blood being withdrawn through a catheter placed in the right hepatic vein. The correlation of these results with the suprahepatic studies was good. In an additional five patients, a comparison was made with the BSP method for estimating hepatic blood flow.

A detailed description of the technique will be presented and will include a discussion of the possible sources of error that can occur in the method. From the general dilution curves inscribed, differential circulation times from

spleen to liver, from spleen to hepatic vein, and spleen to brachial artery were also obtained. In the latter instance a second scintillation probe counter was placed over the antecubital fossa.

A decrease in the hepatic blood flow was obtained following forced exercise, while the ingestion of food increased the blood flow. The intravenous injection of Pituitrin in sufficient amounts to produce marked skin pallor decreased the hepatic blood flow by approximately 40 per cent.

It is to be emphasized that the technique of intrasplenic injection used in this study would appear to be innocuous. In the fifty patients studied, many of whom had two and not infrequently three splenic punctures, no untoward reactions or complications occurred.

115. STRUCTURE AND FUNCTION OF HEART MITOCHONDRIA IN EXPERIMENTAL CIRCULATORY SHOCK

JOSEPH G. STRAWITZ, M.D., AND HELEN HIFT, PH.D., MADISON, WIS.

(INTRODUCED BY CHARLES W. CRUMPTON, M.D.)

Mitochondria are known to be responsible for the oxidative and energy-producing activities of functioning cells. They have been studied morphologically and enzymatically in an effort to characterize changes which may occur in the failing heart of hemorrhagic shock.

Irreversible shock was produced in dogs by using a modification of the Wiggers technique. An average of 45 per cent of the theoretically calculated blood volume was removed to produce a MABP of 40 mm. Hg for 3-5 hours. The left ventricular musculature was removed at the end of the shock period. The shock animal and a control dog were sacrificed within 15 minutes of each other and the mitochondrial fraction of the hearts was prepared by differential centrifugation and tested simultaneously. The ability of these preparations to oxidize Krebs cycle substrates and to form ATP was determined by using the Warburg technique and suitable spectrophotometric methods. Simultaneous mitochondrial morphology was studied under phase contrast following homogenization and fixation.

The results indicate that heart mitochondria are structurally altered in circulatory failure induced by hemorrhagic shock. Normal mitochondria in free-floating suspension show square and spindle-shaped rods with moderately translucent centers. The particles observed in preparations from shocked dog heart are swollen, and irregular in shape with indistinct boundaries and translucent centers. Harmon observed a series of transformations of normal mammalian mitochondria when allowed to stand in sucrose suspensions. The normal rod formed a sphere and finally swelled into a large balloon form which he called "crescents." The micotchondria prepared from shocked dog heart underwent these changes less readily. Those crescents formed were small and compact as compared to the normal.

Water extracts derived from the mitochondria of shocked dogs generally contained more protein than similar normal preparations and higher extinctions especially in the flavin and ultraviolet regions. The oxidation of Krebs cycle intermediates was essentially unimpaired even after 5 hours of shock. No requirement for coenzymes could be demonstrated. However, in the presence of the hexokinase system, oxygen uptake and phosphate esterification were decreased.

These results suggest a change in mitochondrial structure accompanied by a defect in oxidative phosphorylation (ATP formation).

116. THE EFFECT OF EXERCISE ON THE PULMONARY BLOOD FLOW IN PATIENTS WITH LEFT-TO-RIGHT SHUNTS

H. J. C. Swan, M.B., Ph.D. (By Invitation), Hiram W. Marshall, M.D. (By Invitation), and Earl H. Wood, M.D., Ph.D., Rochester, Minn.

Knowledge concerning adaptive circulatory changes in patients with left-to-right shunts is lacking. In exploration of this question, a total of 15 patients—four with patent ductus arteriosus, four with ventricular septal defect, and seven with atrial septal defect—were studied in the horizontal position by the cardiac catheterization technique before and during exercise on a bicycle ergometer.

In six patients with equal aortic and pulmonary artery pressures, the average pulmonary blood flow (liters per minute per square meter) increased from 6.6 before to 7.7 during exercise; but this was not statistically significant. The systemic blood flow rose from 2.5 to 5.7 liters/min./m.^{2} and the left-to-right shunt (pulmonary flow − systemic flow ÷ pulmonary flow) decreased from 60 to 25 per cent. The pulmonary artery pressure rose from 94 mm. of mercury systolic and 45 diastolic to 126 systolic and 61 diastolic. The total pulmonary resistance did not change significantly.

In seven patients without pulmonary hypertension (pulmonary artery systolic pressure less than 50 mm. Hg.), the pulmonary blood flow during rest was above the range of normal at a group average of 9.1 liters/min./m.^{2} before, and showed an insignificant increase of 1.0 liters/min./m.^{2} during exercise, while the systemic flow increased from 3.5 to 4.7 liters/min./m.^{2} The left-to-right shunt declined from 60 per cent to 43. The pulmonary artery pressure and pulmonary resistance were essentially unchanged. In the two patients whose blood flow during rest was less than the extreme of normal (5.7 liters/min./m.^{2}), the average pulmonary blood flow of 3.5 during rest was increased during exercise to 5.5.

Patients who have sizable left-to-right shunts with or without pulmonary hypertension appear to respond to the stimulus of moderate exertion by maintaining the pulmonary blood flow at a relatively unchanged level and by substantially decreasing the proportion of blood shunted left to right. From the general circulatory standpoint, the right side of the heart then works in a more efficient manner. These findings may explain the relatively good tolerance to exercise which is shown by many patients with large left-to-right shunts.

117. ALTERATIONS IN THE NUTRITIONAL REQUIREMENTS OF CULTURED FIBROBLASTS

H. E. Swim, Ph.D. (By Invitation), and R. F. Parker, M.D., Cleveland, Ohio

Sixty strains of fibroblasts from human and rabbit tissues were established in vitro. All except five ceased to proliferate after an interval of rapid growth which varied from three to thirty subcultures and was a function of the origin of the culture, medium, and culture methods employed. Of the strains which continue to proliferate after three years, two (human foreskin and uterus; U12) became altered morphologically and physiologically, whereas three strains from rabbit tissues are indistinguishable, by current cultural criteria, from other strains derived from normal tissues of this species except for their ability to proliferate indefinitely. One interpretation of these findings, with regard to the origin of permanent cell strains, is that a small proportion of cells were altered sufficiently by mutation to be capable of sustained growth

in the media employed. This conclusion was supported by studies with newly established strains which indicated their nutritional requirements changed progressively on serial cultivation. The possibility that the nutritional characteristics of cells could be altered in response to variations in the composition of the medium was examined in more detail by employing strain L (mouse fibroblasts) which was derived from a single cell and strain U12. The composition of the regular medium (synthetic medium 703 plus embryo extract and serum) was changed by eliminating either the serum or embryo extract. In each case, growth was reduced markedly, the cultures became progressively necrotic until it appeared that most cells were involved. After many weeks, a few healthy cells were observed in the cultures and their progeny yielded strains which proliferate rapidly in the new media. The growth response of these sublines of L and U12 in a variety of media indicate conclusively that their nutritional requirements differ significantly from those of the original strains. These data will be discussed in relation to the propagation of normal fibroblasts and their use in studies with viruses. The possible relationships between cellular alterations in vitro and those occurring in vivo will also be considered.

118. THE COLLECTION OF LYMPH FROM INTACT PERIPHERAL CUTANEOUS LYMPHATICS OF MAN

SAM A. THREEFOOT, M.D., NEW ORLEANS, LA.

During investigations of the peripheral lymphatics in congestive heart failure, a method for collection and study of lymph from intact cutaneous lymphatics was developed.

After visualization of lymphatic channels by the intradermal injection of 0.1 ml. of 11 per cent aqueous Patent Blue V or 4 per cent Direct Sky Blue, a 23 gauge or a 30 gauge needle, modified so that plastic tubing with an internal diameter of 23 gauge can be snugly attached, is inserted into a stained lymphatic in the manner of a venipuncture. Appearance of stained lymph in the plastic tubing during approximately five minutes of aspiration with a syringe ascertains success of the cannulation. Continued flow of lymph into the tubing is maintained by steady aspiration at −400 mm. of water.

Lymphatics in forearms or legs were cannulated 57 times in 25 studies. In 23 cannulations sufficient lymph was obtained for quantification by weighing the tube before and after the lymph was expelled. Amounts collected ranged from 0.15 to 19.1 mg. Rates of collection varied from 0.0038 mg./min. to 0.468 mg./min.) (mean 0.156). These rates were more rapid from edematous subjects (mean 0.24 mg./min.) than from nonedematous ones (mean 0.08 mg./min.). Collection was slightly more rapid from legs than from forearms. This is not a measure of rates of flow within the lymphatic channels.

Intralymphatic pressure was estimated to be 20-30 mm. of water in one control subject and 50-75 mm. of water in one subject with lymphedema and venous disease.

Sodium concentration was determined by flame photometry after dilution in siliconed containers thoroughly rinsed with triple distilled water to minimize contamination of the small sample. In 9 samples from 6 subjects, the mean lymph sodium :serum sodium ratio was 1.026 (S.D. ±0.056).

When small volume is not a limiting factor, the fluid collected can be studied for other chemical, isotopic, or cytologic contents.

119. CLINICAL EVALUATION OF TOLBUTAMIDE (1-Butyl-3-p-Tolylsulfonylurea) IN THE TREATMENT OF DIABETES MELLITUS

ROGER H. UNGER, M.D., DALLAS, TEX.

(INTRODUCED BY A. I. BRAUDE, M.D.)

A clinical evaluation of tolbutamide (1-butyl-3-p-tolylsulfonylurea) was undertaken in 20 diabetic patients, who were classified into three groups: I. Severe labile diabetics. II. Moderately severe stable diabetics in whom insulin had been required for adequate control. III. Milder stable diabetics controllable with varying degrees of carbohydrate restriction without insulin.

Base-line studies were conducted for at least seven days prior to beginning therapy. During this control period and throughout the study, labile diabetics were maintained on suboptimal doses of insulin, while all stable diabetics were fed constant, calorically adequate, regular diets and received no insulin. After adequate base-line studies tolbutamide therapy was begun with daily oral doses of 3 Gm. In unresponsive patients, dosages were gradually raised to as high as 12 Gm. per day.

Group I. (3 patients): In no case was improvement observed during tolbutamide therapy. Profound insulin reactions occurred in one patient despite progressive deterioration in control of diabetes. *Group II. (8 patients):* Decrease in fasting hyperglycemia and suppression of glycosuria was observed in only 3 patients. *Group III. (9 patients):* Decrease in fasting hyperglycemia and suppression of glycosuria occurred in all 9 patients.

Untoward effects were encountered in two subjects. One patient developed urticaria, and generalized erythema occurred in another. In both, spontaneous clearing took place without termination of therapy. Liver function tests, urinalyses, complete blood counts, and I^{131} uptake studies failed to reveal significant alterations.

The results confirm the effectiveness of tolbutamide in regulating many patients with mild and moderately severe stable diabetes, particularly the milder cases controllable with diet alone. Among the labile diabetics, not only was no benefit observed, but the concomitant administration of tolbutamide and insulin may possibly have been a factor in inducing hypoglycemic episodes.

120. CHRONIC RENAL DISEASE IN CHILDREN—THE RENAL LESION BY LIGHT AND ELECTRON MICROSCOPY

ROBERT L. VERNIER, M.D. (BY INVITATION), MARILYN G. FARQUHAR, PH.D. (BY INVITATION), JOEL G. BRUNSON, M.D. (BY INVITATION), AND ROBERT A. GOOD, M.D., MINNEAPOLIS, MINN.

The etiology, pathogenesis, and interrelationships between acute and chronic glomerulonephritis, the nephrotic syndrome, the nephritis of anaphylactoid purpura, and the nephritis of disseminated lupus erythematosus are not understood. In an attempt to gain insight into these problems, an extensive study of 60 children with chronic renal disease was undertaken.

Clinical and laboratory studies, renal function tests, and renal biopsies were obtained. The renal tissue was studied by light and electron microscopy. In this series the various disease processes had been active from two weeks to seven years.

The light microscopic studies fail to demonstrate a clearly distinguishing lesion of the glomerulus in the various disorders, since at a given stage of progression of the disease the light microscopic findings may be very similar.

Electron microscopy revealed a characteristic abnormality of the epithelial cell of the glomerular capillary in the biopsies of all the nephrotic children. The glomerular lesion of lupus erythematosus is characterized by a thickening of the lamina densa or basement membrane proper. An abnormality of the endothelial cell layer of the glomerular capillary wall was found to be the early lesion of subacute or chronic glomerulonephritis. A preliminary classification of the glomerular lesions of the chronic renal diseases as revealed by the electron microscope will be presented.

121. THE EFFECT OF HEPARIN ON FIBRINOLYSIS

K. N. von Kaulla, M.D., S. T. McDonald, B.S., and G. H. Taylor, Denver, Colo.

(Introduced by Matthew Block, Ph.D., M.D.)

The induction of fibrinolysis as therapy of thromboembolic disorders made the investigation of the action of heparin on fibrinolysis of importance, since fibrinolysis does not eliminate hypercoagulability when present and may even enhance it.

The test arrangement consisted of heparin (Connaught) made up to concentrations of 0.525 (I), 0.125 (II), 0.025 (III), 0.005 (IV), 0.001 (V) mg./ml. in 0.01, 0.05, and 0.1 per cent solutions of urofibrinolysokinase in (1) buffered saline, (2) plasma euglobulin, or (3) citrated human plasma, respectively. The rate of fibrinolytic activity was obtained by placing 0.03 ml. of the various mixtures on the surface of fibrin plate and the area of the lysed zone was measured at intervals.

A. Bovine plates (0.6 per cent bovine fraction I): In all of the test concentrations in saline or euglobulins, heparin had no effect on the lytic activity. However, heparin concentrations I and II in citrated human plasma produced delay of onset and reduction in rate of lysis, on both heated and unheated plates. There was occasionally enhancement of lysis with smaller amounts of heparin (IV and V). The extent of heparin interference with fibrinolysis varied from plasma to plasma and from lot to lot of fibrinogen.

B. Human plasma plates, using plasma from subjects exhibiting induced fibrinolysis by intravenous injection of 300 μg of a pyogenic lipopolysaccharide (1,083): (1) There was a stronger over-all fibrinolytic activity on unheated plates made from the patients' native preinjection plasma than on bovine plates. (2) Heparin concentrations I and II produced marked inhibition of pyrogen-induced fibrinolytic activity of plasma on both types of bovine plates. (3) Surface measurement of the lysed area of plasma plates showed no appreciable differences, their volumetric determination revealed about 20 per cent more liquefaction with heparin concentrations III and IV compared with the heparin-free control after several hours' incubation. (4) Corresponding results were obtained by testing urofibrinolysokinase-activated plasma on plasma plates of the same subject suggesting an enhancement of fibrinolysis by heparin in the presence of human substrates and enzymes. These results suggest that repeated injection of small doses of heparin might enhance rather than inhibit fibrinolysis.

121a. FEVER OF UNKNOWN ORIGIN OF ELEVEN YEARS' DURATION IN A LAD TWELVE YEARS OLD

BURTON ARMIN WAISBREN, M.D., MILWAUKEE, WIS.

The patient is a twelve-year-old boy who first began to run a septic fever at the age of six months that was associated with draining abscesses and "blisters" over his body. The septic fever continued with a remission never longer than six months, for the next ten years. History revealed exposure to a farm environment at the age of four months, a generalized skin rash at the age of six months, probable response of the fever to iodides and sulfadiazine, multiple draining sinuses through the abdominal wall, six bouts of severe pneumonia, intolerance to penicillin and the tetracyclines, and in spite of all of this normal growth and development. Examinations had revealed high spiking temperatures, multiple calcifications throughout his liver, and nodular red skin lesions over the extensor surfaces of his arms and legs. The significant negative results of extensive laboratory studies were negative tests for human and avian tuberculosis, normal gamma globulin and properdin levels, and negative skin tests for histoplasmosis, blastomycosis, and coccidioidomycosis. Significant positive laboratory findings were positive blood, bone marrow, and stool cultures for Salmonella enteritidis, and consistently positive complement fixation titers (1:32) against blastomycosis. Serial chest x-rays showed rather evanescent pulmonary infiltrations. Eighteen months ago the patient became afebrile after an extensive course of chloramphenicol and since that time six exacerbations of illness have apparently responded to this antibiotic. The patient now is being given a daily dose of chloramphenicol and remains asymptomatic and afebrile at the present time. The case will be discussed in the light of our experience regarding the natural history of blastomycosis and a limited experience with chronic Salmonella infections. The questions to be asked are:

1. Does the boy have both salmonellosis and blastomycosis?
2. Has he had chronic salmonellosis for twelve years?
3. What should we do next?

122. THE CLINICAL SIGNIFICANCE OF SERUM PROTEIN CHANGES IN HODGKIN'S DISEASE INCLUDING THE PHENOMENON OF "ANTICIPATION"

ROBERT L. WALL, M.D., COLUMBUS, OHIO

Frequent serial observation of the serum proteins of patients with Hodgkin's disease by zone electrophoresis has shown pattern distribution changes characteristic of active Hodgkin's disease and conversely quite different for inactive Hodgkin's disease. A series of over 140 patients with this disease have been followed at University Hospital in many instances for over three years. The changes characterizing the active stage of the disease are (1) significant increases in alpha$_2$ globulin, (2) "splitting" of beta globulins, (3) increases of gamma globulins. In 600 serum analyses on this group of patients these protein changes correlated with clinical and radiographic evidence of Hodgkin's disease activity. A large number of inactive Hodgkin's disease patients conversely failed to show these changes. In isolated cases these changes preceded clinical evidence of Hodgkin's reactivation by six to nine months. In comparison with the erythrocyte sedimentation rate and C-reactive protein tests these electrophoretic changes have been more specific and sensitive.

The importance of early and vigorous treatment as soon as the disease is reactivated is obvious. Therefore, in a histologically proved case of Hodgkin's disease, relapse heralded first by these changes of serum proteins will facilitate prompt treatment, possibly before obvious node masses become clinically apparent.

The nonspecificity of these serum protein electrophoretic changes are recognized and comparison is made with other patients whose serum proteins have been examined and correlated with their clinical diagnosis.

123. AVR—A CLUE TO RIGHT VENTRICULAR PREPONDERANCE

RICHARD H. WASSERBURGER, M.D., AND JOHN B. BROWN, M.D.,
MADISON, WIS.

(INTRODUCED BY GEORGE G. ROWE, M.D.)

Electrocardiographic data on 1,000 normal infants and young children have been critically analyzed so as to establish the range of normal. RsR′ patterns with predominant R′ waves as well as predominant pure R-waves were not uncommon in the right precordial chest leads of this group. These normal patterns simulated accepted criteria of right ventricular preponderance. Augmented limb lead, AVR, however, offered a more stable QRS complex pattern of normalcy. The amplitude of the late R-wave in lead AVR, in the presence of a vertical heart, never exceeded the amplitude of the preceding negative deflection regardless of whether this negative deflection was an S-wave or a Q-wave. The configuration held true regardless of the QRS complex pattern in the right precordial leads.

Having established control criteria, the electrocardiographic patterns of 200 patients with documented congenital heart disease were correlated with the calculated right ventricular work index, the existing right ventricular pressures, and the ratio of the right ventricular work index to the left ventricular work index as determined by cardiac catheterization. The latter ratio has been designated as the ventricular work-load ratio. It was observed that when the ventricular work load ratio approached 0.70, lead AVR consistently revealed a predominant late R-wave. This observation was present in lead AVR regardless of the QRS pattern in the right precordial leads. All cases of congenital heart disease with an increase in ventricular work-load ratio exhibited this pattern.

It is concluded that the most reliable ECG evidence of right ventricular preponderance are: (1) a predominant late R-wave in lead AVR, in the presence of a vertical heart, regardless of the QRS pattern in the right precordial leads, and (2) the presence of an RsR′ pattern with a predominant R′ wave, a pure R-wave, or a qR pattern in the right precordial leads, with evidence of delayed ventricular activation time.

Augmented limb lead AVR is important in detecting significant right ventricular preponderance and in differentiating benign variants of RsR′ patterns in the right precordial leads.

124. AMMONIUM UPTAKE BY THE EXTREMITIES AND BRAIN IN HEPATIC COMA

LESLIE T. WEBSTER, JR., M.D., CLEVELAND, OHIO

(INTRODUCED BY GEORGE J. GABUZDA, M.D.)

In order to clarify the relationship of disordered ammonium metabolism to hepatic coma, ammonium removal from the blood by the extremities and brain was investigated.

Ammonium was determined in fasting blood samples obtained from the peripheral veins, brachial arteries, and superior jugular bulbs in 23 patients

without liver disease (Group I), 18 patients with hepatic cirrhosis (Group II), and 16 patients with cirrhosis and either impending hepatic coma or coma (Group III).

Arterial and jugular bulb blood ammonium concentrations were least in Group I, higher in Group II, and highest in Group III. Ammonium concentrations in blood drawn from these sites correlated better with the clinical classification than did those found in peripheral venous blood.

In patients without liver disease, there was no net removal or release of ammonium by extremities or brain as shown by arterial-venous and arterial-jugular bulb differences. In patients with hepatic cirrhosis (Group II) the extremities, but not the brain, removed ammonium from the blood and this removal was proportional to the arterial blood ammonium concentration. Surprisingly, these patients fell into two distinct groups, one of which removed significantly more ammonium than the other at a given arterial concentration.

Removal of ammonium by the extremities was greater in patients with impending hepatic coma or coma (Group III) than in those without these neurological signs. All Group III patients with gastrointestinal bleeding and four Group III patients without gastrointestinal hemorrhage removed ammonium efficiently at a given arterial concentration. However, seven patients in the latter category demonstrated impaired ammonium removal by the extremities.

In Group III patients with impending hepatic coma or coma, there occurred either a net removal or release of ammonium by brain. A net release of ammonium usually occurred in those Group III patients without gastrointestinal hemorrhage, a net removal usually occurring in those who had evidence of gastrointestinal bleeding.

Although there were individual exceptions, results obtained in these patient groups indicate that the extremities and brain contain ammonium removal mechanisms which operate at increased arterial blood ammonium concentrations. The integrity of these peripheral mechanisms apparently depends upon some function(s) of the liver.

125. A COMPARISON OF SHOCK DUE TO ENDOTOXIN WITH ANAPHYLACTIC SHOCK

MAX H. WEIL, M.D. (BY INVITATION), AND WESLEY W. SPINK, M.D., MINNEAPOLIS, MINN.

The mechanism of shock induced by a single injection of endotoxin has been investigated in the dog employing preparations from *Escherichia coli, Brucella melitensis, Salmonella typhosa,* and *Serratia marcescens.* During this series of investigations, it became increasingly apparent that there was a close similarity between anaphylactic shock in the dog and the shock that followed a single intravenous injection of endotoxin. The following similarities to anaphylactic shock were documented: the progressive clinical manifestations of vomiting and diarrhea, fever, coma, and death; an immediate fall in arterial pressure, elevation of the portal vein pressure, severe congestion of the liver, and the development of extensive hemorrhagic lesions in the small intestine; a rapid and profound fall in white blood cells, thrombocytopenia, and hypoglycemia; and the prompt liberation of a histamine-like substance into the blood stream. Neither anaphylactic shock nor shock produced by endotoxin appears to be due to a direct action on the central nervous system and the administration of adrenergic drugs produces improvement in both cases.

The anaphylactoid response to endotoxin and anaphylactic shock were found to differ in two respects. First, anaphylactic shock is associated with prolongation of blood coagulation attributed to the liberation of heparin. However, no change of the coagulation time could be elicited after endotoxin. Second, anaphylactic shock cannot be produced in the dog after hepatectomy.

In contrast, a gradual fall in arterial pressure and enhanced lethality follow the injection of endotoxin after hepatectomy.

Anaphylaxis is a specific antigen-antibody reaction in a sensitized animal. On the other hand, the anaphylactoid shock observed after injection of endotoxin may be induced by a wide variety of colloidal substances, including inorganic colloidal suspensions, parasitic extracts, snake venom, and peptone. Whether or not the shock produced by these nonspecific agents has a common pathway with anaphylactic shock is not clearly understood. Some of the differences exhibited between shock due to endotoxin and anaphylactic shock do not permit the conclusion at this time that a common pathway is not involved.

126. DESOXYRIBOSE NUCLEIC ACID CONTENT OF HUMAN SPERMATOZOA: INFERTILITY STUDIES

DAVID R. WEIR, M.D., AND CECILIE LEUCHTENBERGER, PH.D. (BY INVITATION), CLEVELAND, OHIO

For 100 consecutive couples from a clinic series of 625, infertility studies were done using the standards of evaluation established by the American Society for the Study of Sterility. Major positive and presumptive diagnoses were as follows; minor defects which could but do not regularly cause infertility are not included: oligospermia 39 per cent; major pathology of Fallopian tubes 11 per cent; anovulation or very irregular ovulation 9 per cent; infertility unexplained 39 per cent; studies not completed 2 per cent.

In previous communications by Leuchtenberger and her associates it has been shown that the desoxyribose nucleic acid (D.N.A.) content of the spermatozoa of fertile men is very constant, whereas it may be variable, usually low, in men suspected of infertility. D.N.A. is measured by the microspectrophotometric technique in individual spermatozoa having a normal morphologic appearance and size. From these preliminary studies it was suspected that abnormal D.N.A. content of sperm could be a factor in the infertility of the 39 couples in our 100 series for whom no positive reason for infertility was discovered. It is to be emphasized that serial semen specimens from these 39 were normal by routine analysis.

For 35 of the 39 men the D.N.A. content of 144 separate semen specimens was determined. The D.N.A. content was normal in only 15 per cent of these 144 specimens, whereas for normally fertile men the D.N.A. is normal for substantially 100 per cent of specimens. Abnormal D.N.A. values were found in all but 2 of the 35 men; one of these men submitted only one specimen, the other two.

These results suggest that the findings in our 100 series may be changed as follows: oligospermia 39 per cent; abnormal D.N.A. 33 per cent; major pathology of Fallopian tubes 11 per cent; anovulation or very irregular ovulation 9 per cent; infertility unexplained 6 per cent; studies not completed 2 per cent. Abnormal D.N.A. content of spermatozoa may be an important cause of sterility or prolonged infertility in otherwise apparently fertile human couples.

127. COMPARISON OF "TES-TAPE" AND CLINISTIX VERSUS CLINITEST UNDER ROUTINE HOSPITAL CONDITIONS

HARRY F. WEISBERG, M.D., AND MARY O. BROWN (BY INVITATION), CHICAGO, ILL.

The new test for urinary glucose utilizing paper strips impregnated with a glucose oxidase enzyme system was compared to a "standard" copper reduction method (Clinitest) which determines total urinary reducing sub-

stances (TURS). Five hundred and ninety-nine hospital urines (routine specimens, glucose tolerance tests, and 24-hour quantitative "sugars") positive with Clinitest were also examined with the "Tes-Tape" and Clinistix glucose oxidase tests. Urines which were Clinitest-negative were not included in the study. The results were not affected by the urine pH which varied from 4.5 to 7.5 with Nitrazine paper. Approximately 21 per cent of the urines positive with Clinitest were negative with Clinistix and Tes-Tape; the Clinitest values for these "negative" urines ranged from trace (0.1-0.3 Gm. per cent) with the largest incidence of 45 per cent to 4 plus (2 Gm. per cent) with an incidence of 6 per cent.

Fifty-nine and one-half per cent of the urines positive by both Clinitest and Tes-Tape had similar "quantitative" values with their respective grading scales; 23.3 per cent of the Tes-Tape results, determining glucose, were much lower than with Clinitest, determining TURS. The Tes-Tape grading scale has a gap between 0.5 and 2 Gm. per cent and the Clinitest scale gap is between 1 and 2 Gm. per cent; 14.1 and 2.7 per cent of the Tes-Tape results were lower and higher, respectively, compared to the Clinitest values between 0.5 and 1.0 Gm. per cent. Only one specimen was higher with Tes-Tape than with Clinitest at concentrations of 0.5 Gm. per cent or less.

Eighty-six of the urine specimens were also titrated with Benedict's quantitative reagent; the majority of the Clinitest and Tes-Tape results were found to be higher than those with quantitative Benedict's.

128. MASSIVE STEROID THERAPY IN RESISTANT THROMBOCYTOPENIC PURPURA

AUSTIN S. WEISBERGER, M.D., LEIF G. SUHRLAND, M.D. (BY INVITATION), AND EDWARD R. ARQUILLA, M.D. (BY INVITATION), CLEVELAND, OHIO

Patients exhibiting persistent thrombocytopenia despite therapy with usual doses of steroids or splenectomy present a difficult problem especially when severe bleeding occurs. This report deals with results obtained in treating such patients with large amounts of Δ_1-hydrocortisone (prednisilone).

Two hundred and fifty to 300 mg. of prednisilone (5 to 10 times the usual therapeutic dose) was administered daily to 10 patients with resistant thrombocytopenic purpura for 10 to 40 days. Eight had previously failed to respond to ordinary amounts of steroid therapy. The platelet count was less than 75,000 per c.mm. in all patients and 6 of the 10 had severe hemorrhagic manifestations. Two patients had idiopathic thrombocytopenic purpura which had not responded to splenectomy. The remaining patients were not considered suitable for splenectomy and included patients with chronic lymphatic leukemia, carcinoma metastatic to bone marrow, toxic suppression of the marrow, disseminated lupus, aplastic anemia, myelophthisic anemia and pancytopenia with apparent absence of megakaryocytes in the marrow.

Six patients responded within 3 to 7 days with an increase in platelets ranging from 300,000 to 1,500,000 platelets per c.mm. Bleeding manifestations subsided promptly. An elective operation procedure was successfully performed in one patient. Three patients relapsed when the dose of prednisilone was decreased to 100 mg. daily. No severe toxic manifestations were encountered.

The four patients who failed to respond to massive steroid therapy were those who had severe marrow depletion. These included two patients with aplastic anemia, one with myelophthisic anemia, and one with absent megakaryocytes.

The mechanism of response was studied by determining the effect of prednisilone on rabbit antihuman platelet antibodies. These antibodies produce agglutination of erythrocytes sensitized with soluble human platelet

antigen in the presence of bis-diazo-benzidene. Such agglutination can be prevented by previously neutralizing the antibody with platelet antigen. Such agglutination is not, however, prevented by previously adding prednisilone. Further studies on the mechanism of action of prednisilone are in progress.

In summary, massive steroid therapy may raise the platelet count in patients with adequate marrow cellularity when ordinary amounts of steroid are ineffective. Such therapy appears to be applicable for emergencies rather than for prolonged maintenance.

129. COENZYME A CONTENT OF RAT LIVER AND KIDNEY FOLLOWING PARTIAL HEPATECTOMY AND ETHIONINE-INDUCED LIVER DAMAGE

ALVIN S. WENNEKER, M.D. (BY INVITATION), AND ROBERT E. SHANK, M.D.,
ST. LOUIS, MO.

Coenzyme A has been demonstrated to have a role in carbohydrate and fat metabolism through catalysis of acetate utilization. Although it has been proposed that this coenzyme may also influence protein synthesis by enhancing the rate of acetate uptake, this relationship has not been conclusively established. It was considered that opportunities existed for exploring this relationship by determination of coenzyme A concentrations in the liver and kidneys of animals in which protein synthesis was markedly stimulated by partial hepatectomy or in which there was interference with utilization of an essential amino acid, methionine, by feeding of ethionine.

Accordingly, coenzyme A has been determined by a modification of the arsenolysis method in kidney and regenerating liver of rats at intervals following surgical removal of two-thirds of the liver mass. A decrease of approximately 30 per cent occurred in concentrations of the coenzyme in the liver during the first three days after hepatectomy with return to control values by the fifth day. In contrast, there was a significant elevation in coenzyme A content of kidney developing within 24 hours of surgery.

Similar determinations were done on the liver and kidney tissue of young rats sacrificed after periods of from 21 to 25 days on a diet containing 0.5 per cent dl-ethionine. There was a significant decrease in hepatic coenzyme A content and a significant increase in renal coenzyme A content in these animals as compared with pair-fed control animals.

The data derived from these experiments do not afford conclusive evidence of an essential role of coenzyme A in protein synthesis. To the contrary the marked depression of liver coenzyme A values after hepatectomy and during the phase of most rapid tissue regeneration and protein synthesis indicate that the coenzyme is not integrally involved. The depressed values in liver associated with ethionine feeding probably result from metabolic inhibition of methionine in systems of synthesis of coenzyme A or of protein. The possible relationships of these findings to clinical states of liver dysfunction or protein deficiency disease will be discussed.

130. THE EFFECT OF BILATERAL ADRENALECTOMY IN A PATIENT WITH MASSIVE ASCITES AND POSTNECROTIC CIRRHOSIS

EMILE E. WERK, JR., M.D. (BY INVITATION), PAUL LARSON, M.D. (BY INVITATION), DAVID W. ELLIOTT, M.D. (BY INVITATION), YOICHI OIKAWA, M.D. (BY INVITATION), JEROME GIUSEFFI, M.D. (BY INVITATION), AND LEON SCHIFF, M.D.,
CINCINNATI, OHIO

Aberrant adrenal steroid metabolism has been demonstrated in hepatic disease and a causal relationship to ascites has been suggested. Because of the

reported benefits of adrenalectomy in the "ascitic" dog, bilateral adrenalectomy was performed in a patient with progressive ascites and postnecrotic cirrhosis. The patient, a man aged 29 years, developed ascites in February, 1955. Therapy included diuretics and paracenteses. In October, 1955, he was admitted to the Veterans Administration Hospital. Despite low sodium diet, diuretics and two paracenteses over the ensuing five months, weight increased from 86 kilograms to 102 kilograms. During March to May, 1956, 13 paracenteses (mean vol. 7,646 ml.) were required to relieve respiratory and pressure symptoms. Urinary output averaged 374 ml./24 hrs. Urinary sodium output was 1.6 meq./24 hrs. (4x).

Bilateral total adrenalectomy was performed uneventfully in June, 1956, employing replacement cortisone (E) and hydrocortisone in the usual fashion. Subsequently, stimulation with ACTH produced no rise in urinary 17-hydroxysteroids or 17-ketosteroids. There was no abrupt diuresis. No paracentesis has thus far been required since operation. Over-all weight was unchanged but weight fluctuations occurred dependent upon E dosage. Weight stabilization or loss, and increased urinary output could be produced by sufficient reduction of E dosage (6-17 day intervals), with only minimal evidence of adrenal insufficiency. Sample data are as follows:

Mean/24 hrs.: 37.5-50 mg. E—weight gain 0.73 kilogram, urinary output 769 ml., urinary sodium 7.8 meq. (1x), B.P. 126/90; 25 mg. E—weight gain 0.45 kilogram, urinary output 772 ml., urinary sodium 100 meq. (2x), B.P. 124/74; 12.5 mg. E—weight constant, urinary output 708 ml., urinary sodium 63 meq. (4x), B.P. 120/70; 8 mg. E—weight loss 0.45 kilogram, urinary output 1,033 ml., urinary sodium 216 meq. (1x), B.P. 116/72, slight weakness, decreased appetite, and depression.

During a recent bout of cellulitis of the legs weight loss and increased urinary output resulted at a higher steroid dosage level.

Final conclusions cannot be drawn. By manipulation of E dosage, further weight loss and diuresis are expected.

131. EFFECTS OF EXERCISE ON PROPERTIES OF THE MYOCARDIUM

William V. Whitehorn, M.D., and Arthur S. Grimmenga, M.S., D.D.S. (By Invitation), Chicago, Ill.

Although effects of exercise on the heart have been widely studied, investigation of direct effects on myocardial properties has not been carried out. Accordingly, in vitro electrical and mechanical properties of left ventricular columna carnae from 13 growing albino rats chronically exercised by swimming to exhaustion were compared with those of 11 littermate controls. Exercised animals showed reduced rate of growth and progressive and significant bradycardia. Ventricle-body weight ratios and adrenal body weight ratios were elevated in exercised animals. Water and protein content of heart and adrenals were normal. Histologic examination revealed no hypertrophy of cardiac fibers in exercised tissue and nuclear counts of unit areas were similar to those of controls.

Exercised myocardial bundles showed significant increase in excitability but refractory periods were not modified. Passive length-tension curves were normal. Exercised strips produced greater tension at given diastolic length than controls and estimated total work capacity was elevated. It is concluded that myocardial changes associated with exercise result in increased ability to perform mechanical work. Effects of exercise upon the heart thus appear to be favorable in the young growing organism.

132. PROLONGED STUDIES OF THE SULFONYLUREAS IN HOSPITALIZED DIABETIC PATIENTS

HENRY L. WILDBERGER, M.D. (BY INVITATION), AND HENRY T. RICKETTS, M.D., CHICAGO, ILL.

In 1955, Achelis and Hardebeck, Franke and Fuchs, and Bertram and associates (Deutsche Medizinsche Wochenschrift **80**: 1449-1460, 1955) published preliminary findings on the hypoglycemic activity of carbutamide (a sulfonylurea) in diabetic patients. Since factors other than the drug (treated tuberculosis, resolving infection, weight reduction, dietary regulation, etc.) could have played a role in ameliorating the diabetes of these patients, it seemed desirable to conduct prolonged studies of otherwise healthy persons with diabetes on a metabolic ward, utilizing adequate control periods and searching for possible side effects.

In preliminary investigations, 3 Gm. of either carbutamide or tolbutamide was administered orally as a single dose to normal ambulatory volunteers. During the ensuing 8 to 24 hours, a mild but significant hypoglycemic effect was observed with both preparations in most, but not all, cases.

Diabetic patients hospitalized for the purpose and diverse in age and in duration, severity, and lability of diabetes were chosen for long-term studies lasting for 6 to 8 weeks. A diabetic woman previously adrenalectomized because of metastatic carcinoma was included.

In general, the following findings of earlier investigators were confirmed: (1) The sulfonylureas proved more effective in the older, obese patients with mild diabetes of recent onset than in the young, thin patients with labile diabetes; in the former group, however, neither carbutamide nor tolbutamide afforded satisfactory control of hyperglycemia and glycosuria in all cases. (2) No significant renal, hepatic, hematologic, neurologic, or endocrine impairment was found by the methods employed.

The adrenalectomized patient, rather than proving unusually sensitive to the medication, showed no detectable effect on carbohydrate metabolism. Urinary 17-ketosteroid levels were unaffected in all patients examined.

Barbiturate potentiation, previously noted in the experimental animal, could not be confirmed in the patient on anticonvulsant sedation.

Continuing studies on out-patients have provided no results at variance with those obtained on the metabolic ward. No hypoglycemic reactions were noted in either group of patients while maintained on the drugs alone.

It is clear that the sulfonylureas cannot be considered a substitute for insulin. Their mode of action remains unknown.

133. OBSERVATIONS ON THE EFFECT OF P-(DI-2-CHLOROETHYL) AMINOPHENYLBUTYRIC ACID (CB 1348) ON LYMPHOSARCOMA AND CHRONIC LYMPHATIC LEUKEMIA

SLOAN J. WILSON, M.D., AND WILLIAM E. LARSEN, M.D. (BY INVITATION), KANSAS CITY, KAN.

CB 1348 (chlorambucil) is a derivative of nitrogen mustard and may be given orally. Observations have been made on 15 patients, 5 with lymphosarcoma and 10 with chronic lymphatic leukemia. In all patients the disease was of long duration. Prior therapy had included the following; x-ray, P^{32}, NH_2, TEM, thio-TEPA, ACTH, and corticosteroids.

Preliminary observations revealed that the bone marrow in each instance was greatly infiltrated with lymphocytes. In all of the leukemic patients there was some degree of thrombocytopenia before therapy. The dosage of CB 1348 was influenced by these observations. The initial daily dose was 6.0 to 8.0 mg. for periods of 27 to 120 days for a total dosage of 108 to 516 mg.

The clinical results were favorable in that the leukocytes decreased in all but one of the patients with leukemia, and in all individuals the enlarged glands and organs decreased in size. One leukemia patient with massive ascites and pleural effusion responded dramatically to CB 1348 after failure of therapy with corticosteroids and thio-TEPA therapy. No complete remission was observed in any leukemic patient. In two leukemia patients the thrombocytopenia progressed (38 and 40,000 mm.^{3}, respectively) and therapy was discontinued. Therapy was discontinued in two patients with lymphosarcoma because of leukopenia (1,000 and 2,700 mm.^{3}, respectively). A marked neutropenia occurred in the patient with 1,000 leukocytes mm.^{3}, the neutrophiles being 8 per cent.

In our experience CB 1348 had a satisfactory margin of safety and disagreeable toxic side effects were not noted. In general the clinical results compare favorably to therapy with NH_2 and TEM.

134. FURTHER OBSERVATIONS ON THE EFFECTS OF A PLASMA LIPID MOBILIZING FACTOR IN MAN

CHRIS J. D. ZARAFONETIS, M.D., GLADYS L. MILLER, M.D. (BY INVITATION), WILLIAM A. STEIGER, M.D. (BY INVITATION), JOSEPH SEIFTER, M.D. (BY INVITATION), DAVID BAEDER, M.D. (BY INVITATION), AND RALPH MYERSON, M.D. (BY INVITATION), PHILADELPHIA, PA.

Steiger and associates recently reported preliminary observations of the effects in man of a plasma dialysate which contains a naturally occurring lipid mobilizing factor (LMF). Marked increases in plasma total cholesterol, total fatty acids, and lipid phosphorus resulted from a single intravenous injection of LMF into each of 25 fasting subjects. In order to characterize further the effects of LMF, studies have been extended to: (1) additional subjects, both fasting and nonfasting; (2) metabolic studies in 4 subjects, and (3) initial tests in man of the purified active principle of LMF.

In the balance studies, patients were maintained for 4 weeks on a liquid formula diet which provided a daily average of 2,230 calories, with an average of 381 Gm. carbohydrate, 166 Gm. protein, and 4.8 Gm. fat. The first and fourth weeks were control periods, while during the second and third weeks, daily intravenous injections of LMF were given in doses increasing from 1.0 to 10 mg./kg. Injection of LMF was followed by a significant rise in plasma cholesterol, total fatty acids, and phospholipids in each subject. These remained elevated for as long as 1 to 2 weeks after the injections of LMF were discontinued. Urinary excretion of cholesterol increased approximately fourfold following administration of LMF, while both fecal and urinary nitrogen excretion decreased, as did the fecal fatty acids and lipid phosphorus.

Finally, in fasting subjects receiving as little as 1.2 meg. of a purified crystalline product of LMF intravenously, there resulted a two- to threefold rise in plasma lipids. The effectiveness of this minute quantity suggests the probable hormonal nature of this material.

135. THE VALUE OF MEAN PRESSURE MEASUREMENTS IN THE PREOPERATIVE DETERMINATION OF REVERSIBILITY OF PULMONARY ARTERY HYPERTENSION IN HIGH-PRESSURE CARDIAC OR VASCULAR COMMUNICATIONS

ROBERT F. ZIEGLER, M.D., EDWARD W. GREEN, M.D. (BY INVITATION), AND RADI MACRUZ, M.D. (BY INVITATION), DETROIT, MICH.

Mean pressure measurements from the low-pressure areas of the central circulation, recorded in a total of approximately 2,000 clinical cases by cardiac

catheterization, were analyzed with special reference to the pulmonary artery wedge (or pulmonary vein)-right atrium gradient ($G_{pw\text{-}ra}$) as an indication of volume-flow versus resistance in the production of pulmonary artery hypertension. It was found that the normal gradient ($G_{pw\text{-}ra}$), independent of the absolute pressure in the right atrium, is approximately 4-5 cm. of saline (3-3½ mm. Hg).

With a physiologically intact interatrial septum $G_{pw\text{-}ra}$ was found to increase in direct proportion to the volume of pulmonary venous return regardless of the level of pulmonary artery pressure. This gradient decreased with obstruction to right ventricular outflow as in pulmonary stenosis and failed to increase proportionately to increased pulmonary artery pressure when this was due predominantly to fixed arteriolar resistance. There was also a decreased $G_{pw\text{-}ra}$ in low-pressure shunts such as interatrial septal defect or anomalous pulmonary venous drainage.

In interventricular septal defect or patent ductus arteriosus with pulmonary artery hypertension there was usually a good correlation between an increase of $G_{pw\text{-}ra}$ and other physiologic and radiographic evidence of increased volume of pulmonary blood flow as well as electrocardiographic evidence of combined right- and left-sided cardiac enlargement. These cases, in contrast to those with pulmonary hypertension due to increased resistance, showed consistent reversibility of their abnormal hemodynamics after surgical correction. Occasionally this gradient was the only finding upon which successful surgery could be predicated, thus further establishing its clinical usefulness.

Problems of instrumentation are discussed briefly.

136. SERUM LACTIC DEHYDROGENASE AND GLUTAMIC OXALOACETIC TRANSAMINASE LEVELS IN DISEASE

H. J. Zimmerman, M.D., M. West, M.D. (By Invitation), and H. G. Weinstein, M.D. (By Invitation), Chicago, Ill.

Recent studies have focused attention on the value of the serum glutamic oxaloacetic transaminase (GOT) level in the diagnosis of myocardial infarction and of hepatic disease. Serum lactic dehydrogenase (L.D.) levels also have been shown to be characteristically elevated in a number of diseases. The possibility that combining these determinations might be of clinical value led to a study of these two serum enzymes in a large group of patients. The serum L.D. level has been determined in 510 patients, the GOT level in 260 patients.

Elevated levels of both enzymes were found in almost all patients with acute myocardial infarction. The L.D. level, while not as markedly elevated as the GOT, was consistently abnormal for 5 to 7 days longer than was the GOT. In patients with hepatic necrosis, whether due to hepatitis, cirrhosis or rarer causes, the GOT was distinctly elevated while L.D. elevation was mild or absent. In several cases of infectious mononucleosis the L.D. elevation was striking, while the GOT elevation was moderate. A similar pattern was seen in patients with megaloblastic anemia. In sickle-cell anemia both enzymes were elevated. In many patients with carcinoma, especially with distant metastases, L.D. levels were increased, but only with marked metastatic involvement of the liver or with obstructive jaundice was the GOT elevated.